THE JOURNAL OF EXPERIMENTAL MEDICINE

THE JOURNAL

OF

EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D.

VOLUME TWENTY-SIXTH

WITH SIXTY-SEVEN PLATES AND ONE HUNDRED AND ONE FIGURES IN THE TEXT

NEW YORK
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
1917

COPYRIGHT, 1917, BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

WAVERLY PRESS
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

ERRATUM.

On page 616, Vol. XXVI, No. 5, November 1, 1917, foot-note 2, for *Marine, D., and Rogoff, J. M., J. Pharm. and Exp. Therap., 1916-17, ix, 1*, read *Stewart, G. N., and Rogoff, J. M., J. Pharm. and Exp. Therap., 1917, x, 1.*

On page 627, foot-note 4, for *Stewart and Rogoff, J. Pharm. and Exp. Therap., 1916-17, ix, 479*, read *Stewart and Rogoff, J. Pharm. and Exp. Therap., 1916, viii, 479.*

On page 638, line 14, for *was interrupted* read *was never interrupted.*

On page 644, line 3, the reference number should be 5 instead of 4.

CONTENTS.

No. 1, JULY 1, 1917.

PAGE

MACNIDER, WILLIAM DEB. A consideration of the relative toxicity of uranium nitrate for animals of different ages. I. Plates 1 and 2 1

MACNIDER, WILLIAM DEB. The efficiency of various diuretics in the acutely nephropathic kidney, protected and unprotected by sodium carbonate. II. Plates 3 to 6 19

PLOTZ, HARRY. Cultivation of *Spirochæta obermeieri*. Plate 7. 37

GATES, FREDERICK L. A valve to regulate the delivery of air and ether vapor in any proportion 41

NOGUCHI, HIDEYO, and KUDO, ROKUSABURO. The relation of mosquitoes and flies to the epidemiology of acute poliomyelitis 49

SMILLIE, WILSON G. New anaerobic methods. Plate 8 59

BLAKE, FRANCIS G. Methods for the determination of pneumococcus types 67

DE BEAUJEU, A. JAUBERT. Cicatrization of wounds. V. New mathematical expression of cicatrization 81

VINCENT, A. Cicatrization of wounds. VI. Bacteriological asepsis of a wound 83

DAUFRESNE, MAURICE. Cicatrization of wounds. VII. The use of chloramine-T paste for the sterilization of wounds 91

CARREL, ALEXIS, and HARTMANN, ALICE. Cicatrization of wounds. VIII. Sterilization of wounds with chloramine-T. 95

BULL, CARROLL G., and PRITCHETT, IDA W. Toxin and antitoxin of and protective inoculation against *Bacillus welchii* 119

No. 2, AUGUST 1, 1917.

FABER, HAROLD KNIEST. Studies in glomerulonephritis. I. A quantitative study of the reaction of the kidney to diphtheria toxin. Plates 9 and 10 139

327906

FABER, HAROLD KNIEST. Studies in glomerulonephritis. II. A form of acute glomerulonephritis produced with diphtheria toxin and *Bacillus coli*. Plates 11 and 12..... 153
PAPPENHEIMER, ALWIN M. Experimental studies upon lymphocytes. II. The action of immune sera upon lymphocytes and small thymus cells. Plate 13..... 163
MCLEAN, FRANKLIN C. The mechanism of urea retention in nephritis..... 181
AUER, JOHN, and GATES, FREDERICK L. Experiments on the causation and amelioration of adrenalin pulmonary edema. Plates 14 to 16..... 201
ROBERTSON, OSWALD H. The effects of experimental plethora on blood production..... 221
KLINE, B. S. Experimental study of organization in lobar pneumonia. Plates 17 to 19..... 239
WALSH, L. S. N. The growth of the ovarian follicle of the guinea pig under normal and pathological conditions..... 245
OPIE, EUGENE L. The relation of apical tuberculosis of adults to the focal tuberculosis of children. Plates 20 and 21..... 263
CARREL, ALEXIS, DU NOÜY, P. LECOMTE, and CARREL, ANNE. Cicatrization of wounds. IX. Influence on the healing of wounds of variations in the osmotic tension of the dressing.. 279

No. 3, SEPTEMBER 1, 1917.

NAKAGAWA, KOAN. Human pulmonary distomiasis caused by *Paragonimus westermanni*. Plates 22 to 31..... 297
KANEKO, RENJIRO, and OKUDA, KIKUZO. The distribution in the human body of *Spirochæta icterohæmorrhagiæ*..... 325
IDO YUTAKA, HOKI, ROKURO, ITO, HIROSHI, and WANI, HIDETSUNE. The rat as a carrier of *Spirochæta icterohæmorrhagiæ*, the causative agent of Weil's disease (spirochætosis icterohæmorrhagica)..... 341
INADA, RYOKICHI. The clinical aspects of spirochætosis icterohæmorrhagica or Weil's disease..... 355
KANEKO, RENJIRO, and OKUDA, KIKUZO. A contribution to the etiology and pathology of rat-bite fever. Plates 32 to 34... 363

IDO, YUTAKA, ITO, HIROSHI, WANI, HIDETSUNE, and OKUDA, KIKUZO. Circulating immunity principles in rat-bite fever 377
MANN, FRANK C. Pulmonary embolism. An experimental study. Plates 35 and 36 387
WRIGHT, J. HOMER, and MINOT, GEORGE R. The viscous metamorphosis of the blood platelets. Plate 37 395
ZINSSER, HANS, and PARKER, JULIA T. Studies on bacterial anaphylaxis and infection. Plates 38 and 39 411
TENBROECK, CARL. Increased virulence of the hog-cholera bacillus produced by passage through rabbits 437
TENBROECK, CARL. The significance of agglutinins in the immunity of the rabbit to the hog-cholera bacillus 441

No. 4, OCTOBER 1, 1917.

COLE, RUFUS. The neutralization of antipneumococcus immune bodies by infected exudates and sera 453
DOCHEZ, A. R., and AVERY, O. T. The elaboration of specific soluble substance by pneumococcus during growth 477
PALMER, WALTER W. Acidosis and acid excretion in pneumonia 495
STILLMAN, ERNEST G. Further studies on the epidemiology of lobar pneumonia 513
COLE, RUFUS, and MOORE, HENRY F. The production of antipneumococcic serum 537
BLAKE, FRANCIS G. Studies on antiblastic immunity 563
ADLER, I. Further studies in experimental atherosclerosis. Plates 40 to 43 581
BULL, CARROLL G. The prophylactic and therapeutic properties of the antitoxin for *Bacillus welchii* 603

No. 5, NOVEMBER 1, 1917.

STEWART, G. N., and ROGOFF, J. M. The relation of the spinal cord to the spontaneous liberation of epinephrin from the adrenals. Plates 44 to 47 613
STEWART, G. N., and ROGOFF, J. M. Effect of stimulation of sensory nerves upon the rate of liberation of epinephrin from the adrenals. Plates 48 to 53 637

NEWCOMER, H. S. Bactericidal fluorescence excited by x-rays. 657
KRITCHEVSKY, I. L. Hemolysins of vegetable origin..... 669
DAVIS, DAVID M., and STONE, HARVEY B. Studies on the development of toxicity in intestinal secretion..... 687
DAVIS, BENJAMIN F., and PETERSEN, WILLIAM F. A comparative study of serum and lymph ferments after feeding.... 693
DAVIS, BENJAMIN F., and PETERSEN, WILLIAM F. A comparative study of lymph and serum ferments during protein shock reactions..... 699
FABER, HAROLD KNIEST, and MURRAY, VIRGINIA. Studies in glomerulonephritis. III. An attempt to produce glomerulonephritis by repeated injections of bacteria. Plate 54.... 707
CLARK, ADMONT H. The interrelation of the surviving heart and pancreas of the dog in sugar metabolism. Second paper.. 721
TAYLOR, EDWARD, and AMOSS, HAROLD L. Carriage of the virus of poliomyelitis, with subsequent development of the infection. Plates 55 and 56..... 745

No. 6, DECEMBER 1, 1917.

STEWART, F. CONSTANCE. A note on Petroff's cultural method for the isolation of tubercle bacilli from sputum and its application to the examination of milk..... 755
COULTER, CALVIN B. The biological identity of the Friedländer bacillus..... 763
ARLITT, ADA HART, and WELLS, H. GIDEON. The effect of alcohol on the reproductive tissues. Plates 57 to 60..... 769
DAVISON, WILBURT C., DAVISON, ATALA S., and MILLER, MILO K. Meningitis in an infant due to a hitherto undescribed organism, *Micrococcus florens*. Plate 61..... 779
GOTO, KINGO. The relation of the spleen to blood destruction and regeneration and to hemolytic jaundice. XVI. The influence of splenectomy and of blood disintegration upon the production of bile pigment..... 795
JONES, F. S., and ARNOLD, J. F. Staggers in sheep in Patagonia. Plates 62 to 65..... 805

GREY, ERNEST G. The diversion of the pancreatice juice from the duodenum into the stomach. Its effects upon the level of gastric acidity and upon the pancreas..... 825
NEWCOMER, H. S. The abiotic action of ultra-violet light. Plate 66..... 841
BURKET, WALTER C. Changes in the peripheral blood consequent upon the diversion of the splenic blood into the general circulation. Plate 67..... 849
BULL, CARROLL G., and PRITCHETT, IDA W. Identity of the toxins of different strains of *Bacillus welchii* and factors influencing their production *in vitro*..... 867
INDEX TO VOLUME XXVI..... 885

THE JOURNAL

OF

EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D.

VOLUME XXVI, NO. 1 JULY 1, 1917

PUBLISHED MONTHLY

AT 2419–21 GREENMOUNT AVE., BALTIMORE, MD.

BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Entered as second-class matter at the Post Office at Baltimore, Md., under the Act of March 3, 1879

Copyright, 1917, by The Rockefeller Institute for Medical Research

WAVERLY PRESS
The Williams & Wilkins Company
Baltimore, U. S. A.

A CONSIDERATION OF THE RELATIVE TOXICITY OF URANIUM NITRATE FOR ANIMALS OF DIFFERENT AGES. I.*

By WILLIAM deB. MacNIDER, M.D.

(From the Laboratory of Pharmacology of the University of North Carolina, Chapel Hill.)

PLATES 1 AND 2.

(Received for publication, January 31, 1917.)

The various ways in which the age of an organism expresses itself has received little consideration in the interpretation of many reactions that occur naturally and that are induced experimentally. The toxic effect of a substance experimentally introduced into an organism is usually interpreted either morphologically, by certain cell changes, or by some alteration in the functional capacity of an organ or a certain group of organs.

In a preliminary note[1] the observation was recorded that animals of different ages showed a variation in their response to the toxic effect of uranium nitrate when the poison was given subcutaneously in a constant quantity per kilo of body weight. The younger animals not only withstood the toxic effect of uranium for a longer period without developing an albuminuria and a glycosuria, but when these animals finally became both albuminuric and glycosuric, the quantitative output of these substances was much less in the younger than in the older animals.

In a more recent paper[2] the observation referred to has been confirmed in a second series of animals. A further observation has been made, that, judging by the time of appearance and the amount of acetone which occurred in the urine,

* Aided by a grant from The Rockefeller Institute for Medical Research.

[1] MacNider, W. deB., On the difference in the response of animals of different ages to a constant quantity of uranium nitrate, *Proc. Soc. Exp. Biol. and Med.*, 1913–14, xi, 159.

[2] MacNider, The inhibition of the toxicity of uranium nitrate by sodium carbonate, and the protection of the kidney acutely nephropathic from uranium from the toxic action of an anesthetic by sodium carbonate, *J. Exp. Med.*, 1916, xxiii, 171.

the older animals gave evidence of developing an acid intoxication much earlier than did the younger ones. In this paper it was also shown that a solution of sodium carbonate given intravenously would protect the kidney of an animal against the toxic effect of uranium, and that it was also possible to protect the kidney of a young animal acutely nephropathic from uranium against the toxic effect of an anesthetic by the use of a solution of sodium carbonate. The ability to furnish such a protection decreased as the age of the animal increased.

It is important to ascertain in a more detailed manner the way in which animals of different ages express their variation to the toxic effect of uranium. The present investigation is therefore primarily concerned with a study of acute uranium intoxications in animals of different ages as indicated by a disturbance in the metabolism of the animals, the severity of which shows a parallel with the age of the animal. During the course of such intoxications the animals become nephropathic. In these animals the kidney has been selected as an organ in which to study the functional and morphological variations which develop during the intoxication. The severity of these changes will be studied in relation to the age of the animal.

EXPERIMENTAL.

Dogs were employed in the experiments. The animals varied in age from pups of 8 months to dogs 8 years of age. The younger animals were raised in the laboratory kennels, while the older animals were obtained from people in the surrounding country who had raised the dogs and could vouch for their age within a few months.

The animals were placed in metabolism cages and given a liberal amount of bread with which was cooked a small amount of meat. The animals received 500 cc. of water daily by stomach tube. After a period of 3 days, which was allowed for normal observations, and during which time animals with a naturally acquired nephropathy or with glucose or acetone bodies in the urine could be excluded, the animals were given subcutaneously on 2 successive days 5 mg. of uranium nitrate per kilo of body weight. In all the animals the uranium intoxication was allowed to persist for 48 hours. At the end of this period the animals were either killed and autopsied, or the nephropathic animals were anesthetized and employed for cer-

tain functional studies which will be reported in Part II of this investigation.

The relative toxicity of uranium for these animals has been investigated by a study of the hydrogen ion content and alkali reserve of the blood, the tension of alveolar air carbon dioxide, and by the time of appearance and quantitative output of acetone and diacetic acid in the urine. The functional capacity of the kidney was ascertained by a study, at different periods during the intoxication, of the time of appearance and total output of phenolsulfonephthalein in the urine and by a study of the urea content of the blood.

The hydrogen ion determinations have been made by the indicator method recently devised by Levy, Rowntree, and Marriott.[3] The alkali reserve of the blood and the determinations of alveolar air carbon dioxide have been made by the methods of Marriott.[4, 5] The quantitative determinations of acetone and diacetic acid in the urine have been made by Folin's[6] method as modified by Hart.[7] The output of diacetic acid is expressed in terms of acetone. The blood urea determinations have been made by the method of Marshall,[8] following the modification suggested by Van Slyke and Cullen.[9] The phenolsulfonephthalein test for kidney function was conducted according to the method outlined by Rowntree and Geraghty.[10]

[3] Levy, R. L., Rowntree, L. G., and Marriott, W. McK., A simplified method for determining variations in the hydrogen ion content of the blood, *Arch. Int. Med.*, 1915, xvi, 389.

[4] Marriott, W. McK., A method for the determination of the alkali reserve of the blood plasma, *Arch. Int. Med.*, 1916, xvii, 840.

[5] Marriott, The determination of alveolar carbon dioxide tension by a simple method, *J. Am. Med. Assn.*, 1916, lxvi, 1594.

[6] Folin, O., On the separate determination of acetone and diacetic acid in diabetic urines, *J. Biol. Chem.*, 1907, iii, 177.

[7] Hart, T. S., On the quantitative determination of acetone in the urine, *J. Biol. Chem.*, 1908, iv, 477.

[8] Marshall, E. K., Jr., A rapid clinical method for the estimation of urea in urine, *J. Biol. Chem.*, 1913, xiv, 283.

[9] Van Slyke, D. D., and Cullen, G. E., A permanent preparation of urease, and its use in the determination of urea, *J. Biol. Chem.*, 1914, xix, 211.

[10] Rowntree, L. G., and Geraghty, J. T., An experimental and clinical study of the functional activity of the kidneys by means of phenolsulphonephthalein, *J. Pharm. and Exp. Therap.*, 1909–10, i, 579.

TABLE I.

Normal Animals.

No. of experiment.	Age.	Weight.	Water in 24 hrs.	Urine in 24 hrs.	Sulfonephthalein.		Blood urea.	P_H.	R. P_H.	Carbon dioxide tension.	Albumin, glucose, acetone, diacetic acid.
					Time of appearance.	Output in 2 hrs.					
	yrs.	*kg.*	*cc.*	*cc.*	*min.*	*per cent*	*per cent*			*mm.*	
1	8 mos.	15.9	500	769	5	68	0.012	7.45	8.0	40	0
2	8 "	10.5	500	509	8	71	0.012	7.4	8.1	40	0
3	1	15.81	500	660			0.015	7.45	8.1	37	0
4	1	19.0	500	960	5	67	0.015	7.4	8.1	43	0
5	2	14.68	500	630			0.015	7.45	8.05	43	0
6	3+	17.01	500	670	5	67	0.015	7.45	8.0	44	0
7	3+	9.8	500	762	4½	67	0.015	7.4	8.0	38	0
8	4+	13.15	500	670			0.015	7.45	8.0	39	0
9	5+	13.3	500	465	4	66	0.012	7.45	8.05	40	0
10	5+	18.2	500	520			0.020	7.45	8.0	40	0
11	8	10.6	500	640	5	73	0.016	7.45	8.0	40	0
12	8+	8.53	500	430			0.015	7.45	8.0	39	0

TABLE II.

Nephropathic Animals after 24 Hours.

No. of experiment.	Age.	Uranium nitrate per kilo.	Urine, 1st 24 hrs.	Sulfonephthalein.		Blood urea.	Acetone per 100 cc.	Diacetic acid per 100 cc.	P_H.	R. P_H.	Carbon dioxide tension.
				Time of appearance.	Output in 2 hrs.						
	yrs.	*mg.*	*cc.*	*min.*	*per cent*	*per cent*	*mg.*	*mg.*			*mm.*
1	8 mos.	5	483	4	64	0.012	0	0	7.45	8.0	38
2	8 "	5	704	5	66	0.012	0	0	7.4	8.1	35
3	1	5	995			0.015	0	0	7.45	8.1	35
4	1	5	745	5	64	0.015	0	0	7.45	8.1	43
5	2	5	459			0.015	0	0	7.45	8.05	35
6	3+	5	1,150	7	54	0.016	0	0	7.35	8.0	40
7	3+	5	435	3½	51	0.015	0	0	7.4	8.0	35
8	4+	5	360			0.015	0	0	7.3	7.95	34
9	5+	5	1,060	8	31	0.015	0	0	7.4	7.95	36
10	5+	5	1,300			0.015	0	0	7.4	7.9	36
11	8	5	645	10	17	0.016	2.6109	3.4812	7.4	7.9	37
12	8+	5	520			0.015	2.6592	2.7559	7.25	7.9	34

TABLE III.

Nephropathic Animals after 48 Hours.

No. of experiment.	Age.	Uranium nitrate per kilo.	Urine, 2nd 24 hrs.	Sulfonephthalein.		Blood urea.	Acetone per 100 cc.	Diacetic acid per 100 cc.	P_H.	R. P_H.	Carbon dioxide tension.
				Time of appearance.	Output in 2 hrs.						
	yrs.	*mg.*	*cc.*	*min.*	*per cent*	*per cent*	*mg.*	*mg.*			*mm.*
1	8 mos.	5	769	5	20	0.012	2.9493	2.2756	7.4	8.0	37
2	8 "	5	509	7	18	0.012	1.3054	4.0614	7.4	8.0	35
3	1	5	660			0.020	3.8680	2.3208	7.4	8.0	35
4	1	5	960	11	21	0.020	2.3691	4.3015	7.35	8.0	39
5	2	5	630			0.020	2.8043	4.1099	7.35	7.9	30
6	3+	5	670	10	24	0.020	1.4010	4.2064	7.35	7.9	35
7	3+	5	762	21	4	0.042	2.3045	4.1092	7.3	7.85	31
8	4+	5	595			0.026	3.7862	4.6416	7.3	7.85	32
9	5+	5	465	20	8	0.022	3.3845	9.6700	7.4	7.9	35
10	5+	5	520			0.022	0.3703	2.2241	7.3	7.85	34
11	8	5	640	20	4	0.030	4.9800	2.2241	7.25	7.85	34
12	8+	5	430			0.030	2.6592	7.3975	7.2	7.8	30

Tables I, II, and III give the normal findings in these animals of different ages for 1 normal day and for the 2 subsequent days of the uranium intoxication. The tables show the results obtained in twelve of the animals which have been selected according to their age as representative of the total number of animals employed in the experiments.

Observations on Normal Animals of Different Ages.

All the animals during the period of 3 days allowed for normal observations were freely diuretic. The total output of urine for the last day of observation varied in the respective animals from a minimum of 430 cc. to a maximum output of 960 cc. The urine was free from albumin, acetone bodies, and glucose, and did not contain casts.

Table I shows that in conducting the sulfonephthalein test for kidney function, the appearance of the dye in the urine varied slightly in the different animals. The earliest appearance was 4 minutes following the injection, while in one animal the appearance of the

dye was delayed for 8 minutes. The total output of the dye in a 2 hour period varied between a minimum of 66 per cent to a maximum output of 73 per cent. These variations have apparently no connection with the age of the animal. The highest output of the dye in this series of animals occurred in an animal 8 years old (Experiment 11, Table I).

The percentage of blood urea has been very constant for animals of all ages. In the normal dog the urea content per 100 cc. of blood has varied between 0.012 to 0.020 per cent.

The hydrogen ion determinations have been made from the oxalated whole blood. In such determinations the hydrogen ion concentration expresses both the volatile and non-volatile acid content of the blood. Recently Marriott[4] has shown that frequently such readings are misleading, and that a more accurate conception of the changes in the hydrogen ion concentration of the blood may be obtained by removing the carbon dioxide from the dialysate, in this way obtaining a reading which represents any change in hydrogen ion concentration which may be due to non-volatile acids. Marriott refers to such a reading as the reserve alkali content of the blood (R. P_H).

In the normal animals of all ages the hydrogen ion concentration of the whole blood (P_H) has varied between 7.3 to 7.45, while the alkali reserve of the blood (R. P_H) has varied between 8.0 to 8.1.

The determinations of the tension of alveolar air carbon dioxide have shown a variation within the limits of normality, 37 to 44 mm. The Marriott method for such determinations was employed in seventy-one dogs and has given remarkably constant results.

From the observations which have been made on the normal animals we may conclude that the animals of different ages show no appreciable difference in their ability to eliminate sulfonephthalein, that the blood urea content in these animals is very constant, and that the animals even though varying much in age show naturally no tendency towards an acid intoxication.

Observations on Animals of Different Ages Intoxicated by Uranium Nitrate.

In the following study of the relative toxicity of uranium, it first became necessary to ascertain whether the weight of the animal, and therefore the total amount of the poison introduced, had any effect in determining the toxicity of the substance. By referring to Tables II and III it will be seen that this factor apparently does not influence the toxic response on the part of the animals. For instance the pup of Experiment 1, with a weight of 15.9 kilos, showed a delayed and slight toxic effect from uranium, while the animal of Experiment 12, 8 years old, and with a weight of only 8.53 kilos, showed clearly the toxic action of uranium during the first 24 hours following the initial injection, and by the end of the second 24 hour period was severely intoxicated. This type of observation has remained constant in all the animals employed in this study.

Following the first injection of uranium all the animals remained freely diuretic. In several of the animals (Experiments 6, 9, and 10) the output of urine was greatly increased.

A study of the sulfonephthalein output by the animals of different ages shows the following variations: There is only a slight change in the time of the appearance of the dye in the urine as compared with the normal animals. The delay in the time of appearance has been most marked in the older animals, while in the youngest animals the time of appearance has either been increased over the normal or remained unchanged.

In the animals of all ages the total elimination of sulfonephthalein in a 2 hour period is reduced. The reduction is slight in the young animals and marked in the old animals. In Experiment 1, a pup 8 months old, the total output of the dye was only reduced from the normal of 68 to 64 per cent. In Experiment 11, an animal 8 years old, a reduction occurred from the normal of 73 to 17 per cent.

Following the second injection of uranium there is a continuation of the relatively greater toxic effect of this poison for the kidneys of the older animals. The youngest animals which have been recorded in Tables II and III were 8 months old and from the same litter. These animals at the end of the 48 hour period of intoxication by

uranium had a sulfonephthalein output of 20 and 18 per cent respectively. The two oldest animals in which sulfonephthalein determinations were made show an output of the dye of 8 and 4 per cent.

The toxic effect of uranium for the kidney as shown by the reduction in the output of sulfonephthalein increases with the age of the animals.

The determinations of blood urea in the pathological as compared with the normal animals enable observations to be made concerning the degree of urea retention in the animals of different ages and also permit a study of the relation between the output of sulfonephthalein by the kidney and the amount of urea retained.

Following the first injection of uranium the percentage of blood urea remained practically constant in all the animals. There was no evidence of a retention of urea even though the output of sulfonephthalein had been greatly reduced. In Experiment 7, Table II, the sulfonephthalein output was reduced to 51 per cent while the percentage of blood urea remained constant. In Experiment 11, the sulfonephthalein output was reduced following the first injection of uranium from 73 to 17 per cent. The percentage of blood urea was uninfluenced by this degree of kidney injury and remained at the normal reading of 0.016 per cent.

Following the second injection of uranium a retention of blood urea was found to occur in all the animals over 8 months old. The animals which show the greatest reduction in the output of sulfonephthalein also show the highest retention of blood urea. The tables of experiments furthermore show that the decrease in the functional capacity of the kidney as indicated by a retention of blood urea increases with the age of the animal.

From these observations it would appear that as compared with the sulfonephthalein test for kidney function, the retention of blood urea is a much later manifestation of kidney inefficiency. When, however, the kidney shows serious impairment of function as indicated by the sulfonephthalein test, there occurs a retention of blood urea which shows a parallel with this test for renal function.

The degree to which the kidney may be impaired and yet show no evidence of a retention of blood urea is illustrated by the first two experiments of Table III. The output of sulfonephthalein by these

two young animals was 20 and 18 per cent, respectively, and yet the percentage of blood urea showed no variation from the normal.

In a previous paper[2] on uranium intoxications in animals of different age it was shown that the time of the appearance of acetone bodies in the urine and the relative amount of these bodies increased with the age of the animal. This observation was interpreted as indicating the development of an acid intoxication, and furthermore to furnish ground for the belief that such an intoxication was more readily induced by uranium in an old animal than in a young animal. This interpretation was strengthened by a series of experiments in which it was found possible to protect an animal against the toxic effect of uranium by the use of an alkaline solution intravenously. The degree of protection conferred by such injections was largely dependent upon the age of the animal. Young animals were more readily protected than were old animals.

Howland and Marriott[11] have recently called attention to the fact that the presence of acetone bodies in the urine is in itself insufficient evidence of a tissue acidosis. It is furthermore well known that in conditions of tissue acidosis the output of these bodies in the urine may be reduced as a result of a decrease in the functional capacity of the kidney. It has therefore seemed advisable in this series of animals to ascertain whether there was any evidence of an acid intoxication other than that shown by the appearance of acetone bodies in the urine. With this object in view determinations of the hydrogen ion content of the blood, the alkali reserve of the blood, and the carbon dioxide tension of alveolar air have been made to determine first, if by these methods any evidence can be obtained of a tissue acidosis, second, if the degree of acid intoxication shows any parallel with the quantitative output of acetone bodies in the urine, and, finally, if the degree of intoxication as indicated by these different methods shows any variation with the age of the animal.

Reference to Table II shows that following the first injection of uranium none of the animals under 8 years of age had a urine which contained acetone or diacetic acid. However, the two oldest ani-

[11] Howland, J., and Marriott, W. McK., A discussion of acidosis. With special reference to that occurring in diseases of children, *Bull. Johns Hopkins Hosp.*, 1916, xxvii, 63.

mals of the series, Experiments 11 and 12, one 8 years old and the other 8 years and a few months old, showed both acetone and diacetic acid in the urine at this early period of the uranium intoxication. By referring to Table II it will also be observed that the appearance of acetone and diacetic acid in the urine of the two oldest animals coincides with the development of other indications of an acid intoxication. These animals also show an increase in the hydrogen ion concentration of the blood, a reduction in the alkali reserve of the blood, and a decrease in the tension of alveolar air carbon dioxide.

A study of the animals of Experiments 8, 9, and 10, which have varied in ages between $4\frac{1}{2}$ and 5 years and 2 months, shows that an acid intoxication may exist without the appearance of acetone bodies in the urine. All these animals had a urine which was free from both acetone and diacetic acid and yet all three of the animals gave other evidence of a beginning acid intoxication. It would therefore seem that the appearance of acetone and diacetic acid in the urine may indicate a beginning acid intoxication from uranium. On the other hand, the absence of these bodies from the urine does not exclude a tissue acidosis, for when other tests are employed such a state may be found to exist.

During the second 24 hours of the uranium intoxication the animals of all ages showed the presence of acetone and diacetic acid in the urine. In so far as the development of an acid intoxication can be determined by the presence of these substances in the urine, at this stage of the intoxication the tendency of the animals to develop an acidosis has extended so as to include not only the old animals but the animals of all ages.

The quantitative output of acetone and diacetic acid shows no constant increase with the increasing age of the animal. In general the combined acetone and diacetic acid output is greater in an old animal than in a young animal, but throughout the series of experiments numerous instances have been observed in which there is no true correlation between the age of the animal and the acetone and diacetic acid content of the urine. For example, in Experiment 2, Table III, in a pup 8 months old, the urine, following the second injection of uranium, contained 1.3054 mg. of acetone and 4.0614

mg. of diacetic acid in terms of acetone per 100 cc. of urine. In Experiment 10, in an animal 5 years and 2 months old, the output of acetone was only 0.3703 mg. per 100 cc. of urine, and the output of diacetic acid which was 2.2241 mg. per 100 cc. was but slightly over half the quantity found in the urine of the young animal.

A study of the relation between the quantitative output of acetone bodies in the urine and the changes in blood and alveolar air shows that when these bodies appear in the urine changes also occur in the blood and alveolar air indicative of a beginning acid intoxication.

The experiments also show that at this stage of the intoxication there is no correlation between the total output of acetone bodies and the other indications of the development of a tissue acidosis. For instance, in the animal of Experiment 10, at the end of the uranium intoxication the hydrogen ion content of the blood had been increased to 7.3, the alkali reserve of the blood was reduced to 7.85, and the tension of carbon dioxide in alveolar air gave a reading of 34 mm. The urine, however, showed a remarkably low output of both acetone and diacetic acid, acetone 0.3703 mg. and diacetic acid 2.2241 mg. per 100 cc. of urine.

From this review of the output of acetone and diacetic acid by animals of different ages intoxicated by uranium, the following conclusions may be drawn. The oldest animals show the toxic effect from uranium by the appearance of these bodies in the urine 24 hours before they appear in the urine of the younger animals. When animals of any age show the presence of these substances in the urine, they also show other evidence of a beginning acid intoxication.

The acid intoxication which develops from uranium cannot be solely ascribed to the formation of acetone bodies. These substances may fail to appear in the urine when there is other evidence of a beginning acid intoxication, and when as shown by the sulfonephthalein test the kidney has not become functionally inactive to such a degree as to cause a retention of these bodies. We must therefore conclude that the acid intoxication which develops from uranium is certainly dependent in part upon the formation or retention of acids other than those of the acetone series. Finally, it has been shown that the quantitative output of acetone and diacetic acid bears no constant relation to the age of the animal, and also that there is no

definite correlation between the quantitative output of acetone bodies and the degree of acid intoxication which may be demonstrated by other tests which have been employed for this purpose.

The variation in the toxicity of uranium for animals of different ages is more clearly expressed in changes in the acid-base equilibrium of the blood than by any other response which has been induced during the course of the intoxication.

Reference to Table II shows that following the first injection of uranium the animals under 4 years of age maintain a hydrogen ion content of the blood which varies only slightly from the normal. None of these animals have shown any change in the alkali reserve of the blood. In contrast with this indication of stability on the part of the animals under 4 years of age, all the animals over 4 years old have not only shown an increase in the hydrogen ion content of the blood but the alkali reserve of the blood has constantly shown a depletion. In these older animals in which the alkali reserve has been reduced, the tension of carbon dioxide in alveolar air has varied between 37 to 34 mm. In the younger animals in which the alkali reserve has remained unaffected the tension of carbon dioxide has varied between 43 and 35 mm.

The relative degree of acid intoxication induced in the animals of different ages is even more clearly shown following the second injection of uranium. By this stage of the intoxication, all the animals over 1 year of age not only show an increase in the hydrogen ion content of the blood, but the alkali reserve of the blood has been severely drawn upon. The younger animals, those under 1 year of age, have either shown no change in the hydrogen ion content of the blood, or in the few experiments in which these readings have varied, the hydrogen ion content has not been increased above 7.4, a point which may be considered within the limit of normal variations. In these younger animals the alkali reserve of the blood has either remained unaffected, or it has not been reduced below 8.0.

A study of Tables II and III not only indicates the differences in the hydrogen ion content and alkali reserve of the blood, which, as has been pointed out, exist between the animals of two age limits, those under 1 year of age and those over 1 year of age, but it also serves to demonstrate that as the animal increases in age there is a pro-

gressive increase in the degree of acidosis. For instance, at the end of the 2nd day of the uranium intoxication the animal of Experiment 6, 3 years and 2 months old, had a hydrogen ion concentration of 7.35 and an alkali reserve of 7.9, while the animal of Experiment 12, 8 years old, had a hydrogen ion content of 7.2 and an alkali reserve of 7.8.

The determinations of carbon dioxide tension in alveolar air of the animals of different ages at the end of the 2nd day of the uranium intoxication show that the older animals that have developed a reduction in the alkali reserve of the blood have an alveolar air carbon dioxide tension which varies between 35 to 30 mm. The younger animals in which no change has taken place in the alkali reserve, or in which the alkali reserve has not been reduced below 8.0, show a tension of carbon dioxide which varies between 39 to 35 mm.

From the observations the conclusion appears clear that one of the constant manifestations of the toxic effect of uranium is the development of an acid intoxication, and that the severity of this intoxication is associated with the age of the animal. The older animals develop a severer intoxication than do the younger animals.

The Pathology of the Kidney in Animals of Different Ages Intoxicated by Uranium.

At the end of the 2nd day of the uranium intoxication, twelve of the animals which have been employed in the experiments were killed and kidney tissue was at once fixed in 10 per cent formalin, Zenker's fluid, and in mercuric chloride-acetic acid to be used in the histological study. The animals from which tissue was obtained varied in age between 8 months, and 7 years and 4 months.

In the foregoing discussion of the relative toxicity of uranium in animals of different ages it has been shown that the toxicity of uranium increases with the age of the animal, and furthermore that the degree of toxicity of this substance is associated with the severity of the acid intoxication which develops in these animals. The following histological study has been undertaken with the object of ascertaining whether there is any pathological difference in the kidneys of animals which have shown very mild grades as contrasted

with severe grades of acid intoxication. The functional tests that have been employed have shown a correlation between the degree of acid intoxication and the ability of the kidney to eliminate sulfonephthalein and urea.

In the histological study it has not been found possible to differentiate between kidneys which were obtained from animals near the same age. In kidneys which were obtained from animals that varied 3 years or more in age there has been found such a difference in the pathological response of the organs as to permit a classification of the kidneys into two groups. The first group is represented by kidneys of animals not over 1 year of age and the second group by kidneys of animals that were over 3 years of age.

The kidneys of the younger animals show no evidence of damage to the vascular tissue. The glomerular capillaries are usually well filled with blood. No exudate has been observed in the subcapsular space or between the tubules. The connective tissue of the kidney has not been edematous. The endothelial nuclei of the glomerular vessels and of the cells lining the capsule of the glomerulus stain normally and show no evidence of degeneration or of proliferation.

The epithelium of the tubules, and especially of the convoluted tubules, shows a definite shrinkage. The lumen of the tubules is prominent and usually free from albuminous material. The nuclei of these cells are large in proportion to the surrounding cytoplasm and stain intensely (Fig. 1).

No stainable fat has been observed in the convoluted tubules. In frozen sections of the kidney stained for fat by Herxheimer's modification of the Scharlach R stain, there has been found in the ascending and descending limb of Henle's loop a very small amount of fat which appears as dust-like particles.

The pathology of the kidney of the older group of animals shows a similarity with the younger group in that no demonstrable changes have taken place in the vascular element of the kidney. The epithelium of the tubules shows the following changes which serve to separate the kidneys of the animals of different ages into the two groups.

The cells of the convoluted tubules show an increase in volume which is variable. In some of the animals the swelling has been marked, while in other animals this change has taken place to a

much less extent. Associated with the swelling of the cells the cytoplasm becomes distinctly granular and occasionally shows vacuolation. The free border of the cells appears ragged. The nuclei are decreased in size and stain less intensely (Fig. 2).

In such cells, unless the animal is very old, no stainable fat has been demonstrated. However, in the loops of Henle the amount of stainable fat has greatly increased over that which has been observed in the younger group of animals. The fat appears as large droplets which frequently coalesce. The difference in the amount of stainable fat in the kidneys of these two groups of animals of different ages is the most constant and striking histological variation.

When these differences in the pathology of the kidney are compared with the variations in the degree of acid intoxication and the alterations in the functional capacity of the kidney that are shown by the two groups of animals, the following differences are found to exist.

The kidneys of the younger group of animals in which the epithelium is histologically well preserved except for the appearance of a slight amount of fat in the tubules of Henle show only slight evidence of a beginning tissue acidosis and the functional capacity of the kidney is much less impaired than is the case with the second group of older animals.

The kidneys of the older animals which give histological evidence of a beginning degeneration of the convoluted tubules and which have shown a marked accumulation of fat in the tubules of Henle, show a much severer grade of acid intoxication than the younger animals, and also show that the functional capacity of the kidney has been severely impaired.

In the older group of animals there is an association between the degree of epithelial injury and the amount of fat in the kidney, with the severity of the acid intoxication, and the extent to which the functional capacity of the kidney has been affected.

As a result of this observation the question naturally arises: Does the kidney injury develop primarily, and the tissue acidosis result from a retention of acid bodies which the kidney is unable to remove, or is the kidney injury secondary to, and dependent upon the acid intoxication, resulting from the administration of uranium?

This question has been in a measure answered in the communication[2] previously referred to. If young animals that are being intoxicated by uranium are given a solution of sodium carbonate intravenously they fail to develop an acid intoxication and the renal epithelium either shows no evidence of degeneration, or the degenerative changes are slight when compared with the epithelial damage which occurs in animals unprotected by the use of an alkali.

CONCLUSIONS.

1. The toxic effect of uranium when given in a constant quantity per kilo of body weight is variable. This variation has been constantly associated with differences in the age of the animals. Uranium is more toxic for an old animal than for a young animal. The establishment of this fact, namely, that the age of an animal may modify the toxicity of a substance, should be taken into account in establishing by animal experiment the degree of activity of substances which are to be used for therapeutic purposes.

2. The toxic effect of uranium nitrate is constantly associated with its ability to induce a tissue acidosis. A severer grade of acidosis is induced in an old animal from uranium than is induced in a young animal.

It would appear that in the response of dogs of different ages to uranium the animals represent a reaction system to this substance which shows an increasing susceptibility as the animal advances from youth to senility.

Insufficient experimental data are as yet available to allow a discussion of the mechanism by which such an acid intoxication is produced.

3. The toxic effect of uranium is manifested locally by certain degenerative changes in the kidney. These changes are more marked in the kidney of an old animal than they are in the kidney of a young animal.

Associated with the severer kidney changes which are especially characterized by a beginning swelling of the renal epithelium and by an accumulation of stainable fat in these cells is the development of a severe grade of tissue acidosis.

4. The functional capacity of the kidney shows a parallel with the degree of acid intoxication and with the severity of the histological changes which have developed in the renal epithelium.

EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. Camera lucida drawing, Leitz oc. 1, obj. 6. The figure is from the kidney of the young dog of Experiment 3. The glomerular vessels are well filled with blood. The epithelium of the convoluted tubules, *a*, shows a distinct shrinkage; the nuclei are large and stain intensely. The lumen of the tubules is prominent. At *b* are shown tubules in which the epithelium is beginning to show an early swelling. The animal gave little evidence of an acid intoxication.

PLATE 2.

FIG. 2. Camera lucida drawing, Leitz oc. 1, obj. 6. The figure is from the old animal of Experiment 11. The glomerulus shows no degenerative changes. The epithelium of the convoluted tubules, *a*, appears granular, stains less intensely than the epithelium of the younger animal of Fig. 1, and the nuclei are small and hypochromatic. The free borders of the cells are ragged. At *b* are shown tubules in which the cells are swollen. At *c* the epithelium shows vacuolation. The animal had developed a severe acid intoxication.

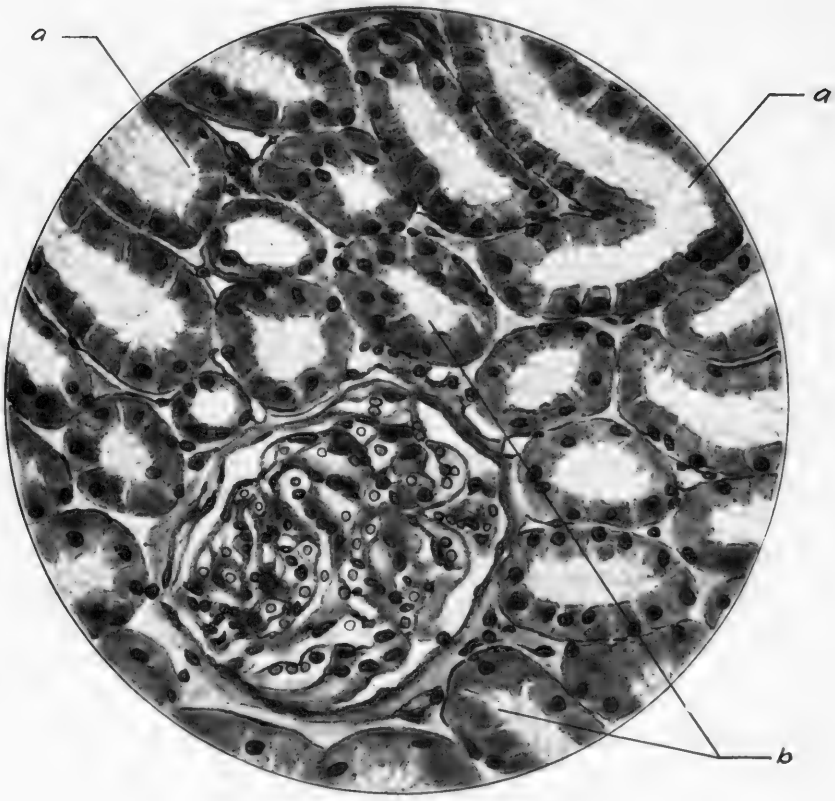


FIG. 1.

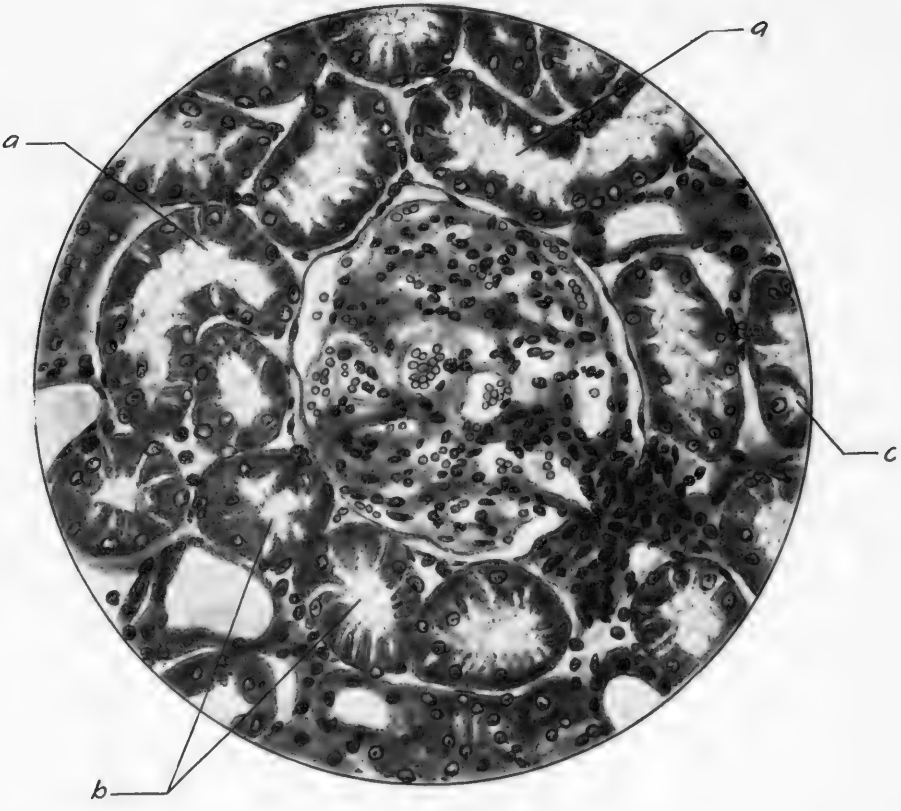


FIG. 2.

(MacNider: Toxicity of uranium. I.)

THE EFFICIENCY OF VARIOUS DIURETICS IN THE ACUTELY NEPHROPATHIC KIDNEY, PROTECTED AND UNPROTECTED BY SODIUM CARBONATE. II.*

By WILLIAM deB. MacNIDER, M.D.

(From the Laboratory of Pharmacology of the University of North Carolina, Chapel Hill.)

Plates 3 to 6.

(Received for publication, January 31, 1917.)

In a recent paper[1] it was shown that intravenous injections of a solution of sodium carbonate inhibited the toxic action of uranium nitrate for the kidney. It was furthermore shown in this paper that the use of such an alkaline solution would protect the kidney of young animals acutely nephropathic from uranium against the toxic effect of an anesthetic. Animals protected in such a manner formed a much larger quantity of urine in a given time limit than did animals of the same age which had received the same amount of uranium nitrate per kilo but which received in place of a solution of carbonate a 0.9 per cent solution of sodium chloride. A histological study of the kidneys of these two groups of animals showed that the animals receiving the carbonate protection failed to develop severe degenerative changes in the renal epithelium, while those animals which were not protected by the carbonate had a renal epithelium which was acutely swollen and undergoing necrosis. In these experiments no information was obtained which would explain the cause of the swelling and necrosis of the epithelium or the relative efficiency of an alkaline solution to furnish a protection against an anesthetic as compared with the efficiency of an equal volume of a solution of sodium chloride.

In Part I of the present study I have been able to show by inducing uranium intoxications in animals of different ages that the severity of the uranium intoxication was dependent upon the degree of acid

* Aided by a grant from The Rockefeller Institute for Medical Research.

[1] MacNider, W. deB., The inhibition of the toxicity of uranium nitrate by sodium carbonate, and the protection of the kidney acutely nephropathic from uranium from the toxic action of an anesthetic by sodium carbonate, *J. Exp. Med.*, 1916, xxiii, 171.

intoxication which developed in the different animals, and furthermore that the older animals developed a severer acid intoxication than did the younger animals.

The object of the present study is twofold: first, to determine the changes in the acid-base equilibrium of the blood induced by an anesthetic in normal animals, in animals acutely nephropathic from a constant quantity of uranium per kilo and protected by the intravenous use of an alkali, and in animals nephropathic from the same quantity of uranium but unprotected by an alkali; second, in such a series of animals to study the efficiency of various diuretics and the relative severity of the pathological changes which have taken place in the kidney.

EXPERIMENTAL.

Forty-six animals were used in this series of experiments. The animals varied in age from pups 8 months old to animals 11 years and 4 months old. Thirty of the animals that were employed in these experiments were used in Part I of this paper. The animals of the present series that are not included in these experiments were subjected to an experimental technique similar to that previously described. Ten of the animals were normal dogs which varied in age from 1 to 5 years. These animals were kept in metabolism cages for 3 days before they were used for experimental purposes, were fed on bread with a small amount of meat, and were given 500 cc. of water daily by stomach tube. The remaining thirty-six nephropathic animals were given the same diet and the same amount of water.

Tables I and II furnish an outline of the observations which have been made on two of the normal animals and thirteen of the acutely nephropathic animals. The animals have been selected according to their age in order to show the influence of this factor in determining the toxicity of an anesthetic and in determining the ability of an alkali to protect the nephropathic kidney against an anesthetic. In the experiments on nephropathic animals, two dogs were used in each experiment. One of the animals received the alkaline solution, while the other animal of the same age received an equal volume of sodium chloride solution and served as a control. On the day of the experiment, prior to the use of an

TABLE I.

No.	Experiment.	Age.	Uranium per kilo on 2 successive days.	Urine on day of experiment.	P_H.	R. P_H.	Carbon dioxide tension.	$NaCO_3$, 3 per cent, or NaCl, 25 cc. per kilo.	Gréhant's anesthetic.	P_H 1st half hr.	R. P_H 1st half hr	Carbon dioxide tension 1st half hr.	Urine flow per min. 1st half hr.
		yrs.	*mg.*	*cc.*			*mm.*		*per cent*			*mm.*	*drops*
1	Normal.	1	0	690	7.45	8.1	39	0	60	7.45	8.1	39	2
2	"	4	0	635	7.45	8.1	43	0	60	7.45	8.1	42	2
3	Carbonate.	8 mos.	5	769	7.25	8.0	34	Na_2CO_3.	60	7.5	8.1	39	8
4	Control.	8 "	5	509	7.4	8.0	35	NaCl equimolecular with Na_2CO_3.	60	7.35	7.95	34	0
5	Carbonate.	11 "	5	723	7.35	8.0	35	Na_2CO_3.	60	7.45	8.2	40	1
6	Control.	11 "	5	765	7.45	8.0	36	NaCl equimolecular with Na_2CO_3.	60	7.4	7.9	32	0
7	Carbonate.	1	5	660	7.4	8.0	34	Na_2CO_3.	60	7.45	8.1	37	7
8	Control.	1	5	960	7.35	8.0	33	NaCl equimolecular with Na_2CO_3.	60	7.3	7.9	30	0
9	Carbonate.	1½	5	575	7.25	7.9	33	Na_2CO_3.	60	7.55	8.2	40	3
10	Control.	1½	5	415	7.35	7.95	33	NaCl equimolecular with Na_2CO_3.	60	7.3	7.9	30	0
11	Carbonate.	3+	5	362	7.3	7.85	31	Na_2CO_3.	60	7.5	8.05	40	0
12	Control.	3+	5	470	7.3	7.9	35	NaCl equimolecular with Na_2CO_3.	60	7.25	7.9	35	0
13	Carbonate.	5+	5	398	7.25	7.85	26	Na_2CO_2.	60	7.35	8.0	45	0
14	"	8	5	340	7.25	7.85	30	"	60	7.5	8.0	45	0
15	"	8	5	310	7.2	7.8	28	"	60	7.45	8.0	45	0

anesthetic, the hydrogen ion content of the blood, the alkali reserve of the blood, and the tension of alveolar air carbon dioxide were determined by the methods previously described. The nephropathic animals were then given intravenously either 25 cc. per kilo of a 3 per cent solution of sodium carbonate or the same amount of a solution of sodium chloride equimolecular with 3 per cent sodium carbonate. The normal animals were not given either of these solutions. In such animals it was possible to obtain information cover-

TABLE II.

No.	Experiment.	Diuretic.	Urine flow per min. 2nd half hr.	P_H 2nd half hr.	R. P_H 2nd half hr.	Urine flow per min. 2nd half hr.	Diuretic solution.	Urine flow per min. 3rd half hr.	P_H 3rd half hr.	R. P_H 3rd half hr.	Carbon dioxide tension 3rd half hr.	Urine flow per min. 3rd half hr.
			drops			*drops*		*drops*			*mm.*	*drops*
1	Normal.	Theobromine, 1%	7	7.4	8.1	3	Urea 0.9%	64	7.45	8.1	38	7
2	"	Pituitrin, 0.5 cc.	10	7.3	8.1	5	NaCl 5%	10 cc.	7.2	8.0	39	5
3	Carbonate.	" 0.5 "	28	7.35	8.1	10	Urea 0.9%	38	7.3	8.0	35	20
4	Control.	" 0.5 "	0	7.35	7.9	0	" 0.9%	0	7.35	7.9	29	0
5	Carbonate.	Theobromine, 1%	4	7.35	8.1	1	NaCl 5%	22	7.35	8.05	37	6
6	Control.	" 1%	0	7.25	7.85	0	" 5%	0	7.25	7.85	21	0
7	Carbonate.	" 1%	12	7.5	8.0	5	" 5%	41	7.3	7.95	30	16
8	Control.	" 1%	0	7.2	7.8	0	" 5%	0	7.2	7.8	27	0
9	Carbonate.	" 1%	13	7.35	8.0	5	Urea 0.9%	44	7.3	8.0	35	21
10	Control.	" 1%	0	7.2	7.8	0	" 0.9%	0	7.1	7.75	23	0
11	Carbonate.	" 1%	0	7.3	7.9	0	NaCl 5%	0	7.25	7.8	29	0
12	Control.	" 1%	0	7.1	7.8	0	" 5%	0	7.05	7.75	24	0
13	Carbonate.	" 1%	0	7.3	7.85	0	" 5%	0	7.2	7.8	26	0
14	"	" 1%	0	7.3	7.9	0	" 5%	0	7.2	7.8	28	0
15	"	" 1%	0	7.3	7.9	0	" 5%	0	7.2	7.8	26	0

ing the natural susceptibility of the kidney to the anesthetic. All the animals were anesthetized by Gréhant's[2] anesthetic in 60 per cent strength. When the animals had become completely anesthetized the abdomen was opened and cannulas were tied into both ureters. The flow of urine was recorded in drops per minute. Half an hour after giving the anesthetic observations were made on the acid-base equilibrium of the blood and these observations were repeated every half hour during the experiment. The experiments were terminated at the end of $1\frac{1}{2}$ hours. At each half hour period during the experiments the animals were given, intravenously, one of the following diuretic solutions, a record being made of the relative efficiency of these substances in the different groups of animals: theobromine, 1 per cent, 1 cc. per kilo; pituitrin (Parke, Davis and

[2] The animal is given 0.25 cc. per kilo of a 4 per cent solution of morphine. This is followed in half an hour by 10 cc. per kilo of the following mixture: chloroform, 50 cc.; alcohol and water, each 500 cc.

Company), 0.5 cc. regardless of weight; urea solution, 0.9 per cent, in 0.9 per cent sodium chloride, 10 cc. per kilo; sodium chloride, 5 per cent, 10 cc. per kilo.

The Efficiency of Diuretics in the Normal Animal Anesthetized by Gréhant's Anesthetic.

In Experiments 1 and 2 of Tables I and II an outline is given of the blood changes, the changes in carbon dioxide tension, and the efficiency of several types of diuretic substances in two of the ten normal animals which were anesthetized with Gréhant's anesthetic. The animals were respectively 1 and 4 years old. Both these animals were freely diuretic prior to the anesthetic. The hydrogen ion content of the blood, the alkali reserve, and the tension of carbon dioxide in alveolar air were normal. Half an hour after the animals were anesthetized no change had taken place in these readings except a reduction of the tension of carbon dioxide of 1 mm. The urine flow from each animal was two drops per minute. The animal of Experiment 1 was given 1 cc. per kilo of a 1 per cent solution of theobromine, while the animal of Experiment 2 received 0.5 cc. of pituitrin. The flow of urine was increased by theobromine to seven drops per minute and by pituitrin to ten drops per minute. At the end of the second half hour period no change had taken place in the alkali reserve of the blood. The combined volatile and non-volatile acid content of the blood as indicated by the hydrogen ion determinations had increased in the animal 1 year old from 7.45 to 7.4, while in the animal 4 years old this reading was 7.3, as compared with the normal of 7.45. At this stage of the anesthesia the animal of Experiment 1 was given 10 cc. per kilo of a 0.9 per cent solution of urea, and the animal of Experiment 2, 10 cc. per kilo of a 5 per cent solution of sodium chloride. The diuresis from both these substances was marked. The urea solution increased the flow of urine to sixty-four drops per minute and the sodium chloride solution to 10 cc. per minute. At the end of the experiments, $1\frac{1}{2}$ hours after the animals were anesthetized, the flow of urine from both animals was in excess of the normal. In the young animal of Experiment 1 no change had taken place during the period of anesthesia in the hydrogen ion con-

tent or alkali reserve of the blood and practically no change in the tension of alveolar air carbon dioxide. The tension had been reduced 1 mm. In the animal of Experiment 2, 4 years old, the hydrogen ion content had increased from the normal of 7.45 to 7.2 and the alkali reserve showed a reduction from 8.1 to 8.0. The tension of carbon dioxide had decreased from the normal of 43 to 39 mm.

The experiments on the normal animals show that the normal kidney is relatively non-susceptible to the toxic effect of Gréhant's anesthetic. Animals anesthetized by this mixture, the active anesthetic ingredients of which are chloroform and alcohol, remain diuretic during a period of $1\frac{1}{2}$ hours and respond to such diuretic substances as theobromine, pituitrin, and solutions of urea and sodium chloride. The experiments also indicate that in the older normal animals there is a tendency for the anesthetic to increase the hydrogen ion content of the blood and reduce the alkali reserve of the blood. The extent to which these changes have developed in the normal animals has not been indicated by any reduction in the response of the kidneys to various diuretics.

At the end of the experiments on normal animals and prior to the death of the animals the kidneys were removed and studied histologically. The findings were negative.

The Efficiency of Diuretics in Nephropathic Animals Anesthetized by Gréhant's Anesthetic and Protected against the Anesthetic by a Sodium Carbonate Solution as Compared with Animals of the Same Age Which Were Unprotected.

Thirteen of the thirty-six experiments on nephropathic animals are included in Tables I and II. Ten of the experiments were conducted in pairs, one animal receiving intravenously prior to the anesthetic 25 cc. per kilo of a 3 per cent solution of sodium carbonate, while the other animal which was used as a control was given an equivalent volume per kilo of a solution of sodium chloride equimolecular with the carbonate solution. All the animals had been intoxicated by uranium in the dose of 5 mg. per kilo which was given on the 2 days prior to the day of the experiment. During the last day of the uranium intoxication all the animals were diuretic. The output of urine varied between a minimum of 310 cc. to a maximum

of 960 cc. The urine was collected at 9 a.m. and the animals were anesthetized for the experiments in the afternoon of the same day. At this time the hydrogen ion and alkali reserve determinations were made. These determinations have therefore no association with the output of urine as recorded in Table I. It will, however, be noted that the older animals with a well marked acid intoxication show a decrease in the output of urine.

A study of the animals included in Table I, before they were anesthetized, confirms the observation made in Part I of this investigation. The older animals at the end of a 2 day period of intoxication by uranium show a severer grade of acid intoxication than do the younger animals. The animals over $1\frac{1}{2}$ years of age show a more constant increase in the hydrogen ion content of the blood, and the alkali reserve of the blood has been drawn upon to a greater extent than has been the case with the animals under $1\frac{1}{2}$ years of age. The carbon dioxide tension shows a greater reduction in the older animals than in the young animals.

Following these determinations the animals were given either the carbonate solution or the solution of sodium chloride, and at once anesthetized with Gréhant's anesthetic in 60 per cent strength. The observations which follow were made at half hour intervals during the experiments.

Reference to the tables of experiments shows that depending upon the relative susceptibility of the kidney to this anesthetic the animals may be divided into two groups. (1) The control animals. All these animals, even though they had first received 25 cc. per kilo of a solution of sodium chloride, became anuric by the end of the first half hour period of the experiment. This applies to animals of all ages. (2) The animals which received a solution of sodium carbonate. These animals may be subdivided into two groups. (*a*) The nephropathic animals not over $1\frac{1}{2}$ years old which received the alkaline solution remained diuretic during the experiments, while (*b*) those animals over this age became anuric and remained anuric during the entire experiment. A study of the changes in the blood and alveolar air of these different groups of animals shows the following variations.

Within half an hour following the anesthetic the control animals

show an increase in the hydrogen ion content of the blood and a beginning reduction in the alkali reserve of the blood. The tension of carbon dioxide either remains constant or is decreased. In the control animals in this short period the anesthetic has increased the degree of acid intoxication which existed prior to the use of the anesthetic. All the animals that received the carbonate solution show at this period of the experiment a decrease in the hydrogen ion content of the blood, an increase in available alkali, and an increase tension of carbon dioxide. Those animals over $1\frac{1}{2}$ years old which resisted the toxic effect of the anesthetic and remained diuretic show a more marked decrease in the hydrogen ion content of the blood and a larger amount of available alkali than do the animals over this age which became anuric from the anesthetic. For instance, in the diuretic animal of Experiment 9 the carbonate solution changed the hydrogen ion reading prior to the anesthetic from 7.25 to 7.55 and the alkali reserve from 7.9 to 8.2. In the anuric animal of Experiment 13 the hydrogen ion determination was changed by the carbonate from 7.25 to 7.35 and the alkali reserve from 7.85 to 8.0. The inability of the alkaline solution to protect the nephropathic kidney against the anesthetic is associated with its failure to decrease the acid intoxication of the anuric animals to the same extent to which the intoxication is modified in the diuretic group.

At this stage of the experiments, the end of the first half hour period, two of the animals were given pituitrin and the remaining eleven animals were given theobromine. The control animals remained uniformly anuric from these diuretic substances. The carbonate-protected animals under $1\frac{1}{2}$ years of age which had not shown a severe acid intoxication prior to the anesthetic and which following the anesthetic and the intravenous injection of the alkaline solution gave no evidence of an acid intoxication were freely diuretic to both pituitrin and theobromine. The older animals, those over $1\frac{1}{2}$ years of age, which prior to the anesthetic had shown a severe grade of acid intoxication and which following the anesthetic and the carbonate solution gave less evidence of protection against the acid intoxication have remained anuric and unresponsive to these diuretic substances. A solution of sodium carbonate is able to protect the kidney of a nephropathic animal against Gréhant's anesthetic up to

a certain age limit. After this age limit is reached the older animals are not afforded a protection by the carbonate but become anuric as do the control animals which have received a solution of sodium chloride.

A study of the tables of experiments from this first half hour period following the anesthetic, through the remaining periods to the termination of the experiments, shows the variations which occur in the acid-base equilibrium of the blood in the control animals, in the carbonate-protected animals which remain diuretic, and in those animals receiving the carbonate which become anuric; it also shows the relative efficiency of diuretics in these three groups of animals.

It will be observed that all the control animals remain anuric through the remaining hour of the respective experiments and that none of these animals show any diuretic effect from a 0.9 per cent solution of urea or a 5 per cent solution of sodium chloride. It will also be observed that in the control animals the degree of acid intoxication tends to increase progressively in severity as the age of the animal increases. The control animal of Experiment 4, 8 months old, at the end of the experiment had a hydrogen ion content of 7.35 and an alkali reserve of 7.9. The carbon dioxide tension was 29 mm. The control animal of Experiment 12, 3 years and 7 months old, had a hydrogen ion content of 7.05, an alkali reserve of 7.75, and a carbon dioxide tension of 24 mm. Nephropathic animals anesthetized by Gréhant's anesthetic and unprotected against the toxic action of the anesthetic by an alkali show the same type of response to the anesthetic as the animals of different ages have shown to uranium. The toxicity of the anesthetic increases with the age of the animal.

During the remainder of the experiments the nephropathic animals which received the sodium carbonate protection permit the same division into two groups. The animals not over $1\frac{1}{2}$ years of age remain diuretic to the conclusion of the experiment, while those over this age remain anuric.

At the end of the second half hour period of the experiments all the animals which had received the carbonate protection were given either 10 cc. per kilo of a 0.9 per cent solution of urea or 10 cc. per kilo of a 5 per cent solution of sodium chloride. The functional response of the kidneys to these diuretics was clear-cut. The younger

group of animals which were previously diuretic to either theobromine or pituitrin and which at the present stage of the experiment showed a hydrogen ion concentration not over 7.35 and an alkali reserve not below 8.0 were freely diuretic to both 5 per cent sodium chloride and a 0.9 per cent solution of urea. The older animals, which earlier in the experiments had shown no diuretic effect from theobromine, at this later stage of the experiment also remained anuric to the solutions of urea and sodium chloride. In this latter group of anuric animals the injected carbonate solution had been rapidly used up so that the blood showed an alkali reserve of not over 7.8.

During the course of the anesthesia all the animals which have received the carbonate protection show an increase in the hydrogen ion content of the blood and a reduction in the alkali reserve. The degree to which these changes take place varies in the diuretic and anuric groups of animals. In the younger group of animals which remain diuretic the reduction in the alkali of the blood takes place gradually so that during an anesthesia lasting $1\frac{1}{2}$ hours the alkali reserve of the blood has not shown a reading below 7.95 and the hydrogen ion content has not been over 7.35. The older group of animals in which no protection was afforded by the sodium carbonate solution and which remained anuric throughout the period of anesthesia have shown a rapid depletion of the blood of the injected alkali, so that by the termination of the experiment this group of animals has had a hydrogen ion content as high as 7.2, and an alkali reserve as low as 7.8.

The changes in carbon dioxide tension in alveolar air of the two groups of animals have shown in general a correlation with the change in the alkali reserve of the blood. In the group of young animals which show less reduction in the alkali reserve the tension of carbon dioxide has varied between 30 to 37 mm. In the older group of animals with a more marked reduction in the alkali reserve the tension of carbon dioxide has varied between 26 to 29 mm.

The foregoing brief review of the differences in the acid-base equilibrium of the blood in the diuretic and anuric groups of animals shows that the establishment of a state of anuria in an animal nephropathic from uranium and anesthetized by Gréhant's anesthetic is associated with the development of a tissue acidosis and with a

failure of the carbonate solution to maintain the reaction of the blood near the point of neutrality.

The Pathology of the Kidney in Nephropathic Animals, Protected and Unprotected against the Toxic Effect of Gréhant's Anesthetic by Sodium Carbonate.

At the termination of the experiments and before the death of the animals the kidneys were removed for histological study. Tissue was fixed in 10 per cent formalin and in mercuric chloride-acetic acid. Sections for routine histological study were stained with eosin and hematoxylin. Frozen sections were stained for fat by Herxheimer's modification of the Scharlach R stain.

Depending upon the severity of the pathological changes which have developed in the kidneys, these organs may be classified into three groups: the kidneys of animals which have received the carbonate protection and which have remained diuretic and responsive to diuretic substances; the kidneys of animals which also received the carbonate protection but which during the development of the anesthesia became anuric and remained unresponsive to diuretic substances; and finally, the group of control animals all of which following the anesthetic became anuric.

The kidneys of the animals which were successfully protected by the carbonate show no degenerative change in the glomerular vessels. These vessels are usually distended with blood and when compared with the glomerular vessels in the anuric group of animals the difference in the degree of distention of the loops is very evident. The epithelium of the convoluted tubules is shrunken. The nuclei of these cells are large and hyperchromatic. The lumen of the tubules is prominent. Occasionally tubules are observed in which the cells show an accumulation of granules, or more rarely vacuoles, and an early swelling. Stainable fat has not been found in the epithelium of the convoluted tubules, the glomerular vessels, or in the connective tissue of the kidney. The loops of Henle usually show a trace of fat (Figs. 1 and 2).

The kidneys of those animals which received the carbonate solution but which were not afforded any protection against the toxic effect

of the anesthetic show a pathological response which is striking when compared with the changes in the kidneys that have been protected by the alkaline solution. The vascular element of the kidney shows no evidence of degeneration. The capillary loops of the glomeruli are certainly not engorged with blood, and when compared with the glomerular vessels of the previous group of animals protected by the carbonate, the glomerular vessels of this anuric group contain less blood. The epithelium of the convoluted tubules is severely swollen. The cell cytoplasm has not become necrotic and the nuclei have not undergone fragmentation. In many of the tubules the lumen has become completely obliterated by the swollen cells (Fig. 3). The epithelium of the convoluted tubules has not contained any clearly defined stainable fat. The loops of Henle show a large amount of fat in the form of droplets, which frequently fuse together.

The kidneys of the control animals, which instead of receiving the alkaline solution were given a solution of sodium chloride equimolecular with the carbonate solution, show a pathological response resembling that of the anuric group of animals which failed to receive any protection from the carbonate solution. The pathological changes in the kidneys of this control group show more advanced evidence of degeneration. The glomerular vessels are not distended with blood. The cells of the convoluted tubules are severely swollen, granular, and frequently hydropic. The nuclei are hypochromatic and undergoing fragmentation. The cytoplasm of many of the cells has become necrotic (Fig. 4). In these cells stainable fat has frequently been demonstrated. The relative amount of fat in the loops of Henle is far greater in this control group of animals which were anuric than in the anuric group of animals that received the carbonate solution.

SUMMARY.

The outline which has been given of the relative toxicity of Gréhant's anesthetic in normal animals, in animals that were nephropathic from uranium and protected against the anesthetic by an alkaline solution, and in those animals which were unprotected by such a solution, furnishes the basis for the following summary.

The kidney of the normal dog is relatively non-susceptible to the

toxic action of Gréhant's anesthetic. The kidneys have failed to show any change in their histological structure during the period of anesthesia. These animals have remained diuretic during the period of anesthesia and have responded to diuretics such as theobromine, pituitrin, and solutions of urea and sodium chloride.

Normal animals anesthetized with Gréhant's mixture for 1½ hours usually show at the end of the experiment either no change, or only a slight variation from the normal, in the hydrogen ion content of the blood, the alkali reserve of the blood, and in the tension of carbon dioxide. In several normal dogs which were over 4 years of age, by the end of an anesthesia of such a duration the animals have shown a reduction in the alkali reserve of the blood and also a decrease from the normal in the carbon dioxide tension of alveolar air. From this observation it would appear that even in a normal animal Gréhant's anesthetic tends to induce an acid intoxication, and as was the case with normal animals which were being intoxicated by uranium, such an intoxication is more readily induced in an old animal than in a young one.

The nephropathic animals which have been anesthetized by Gréhant's anesthetic and in which an attempt has been made to protect these animals against the toxic effect of the anesthetic by the use of a solution of sodium carbonate fall into two clear-cut groups. Those animals of the series not over 1½ years old have shown at the end of the uranium intoxication and prior to the use of the anesthetic a less severe acid intoxication than have the animals of the series which were over 1½ years old. In this younger group of animals the intravenous injection of a 3 per cent solution of sodium carbonate immediately before the animals were anesthetized has succeeded in protecting these animals against the toxic action of the anesthetic. During the following 1½ hours of anesthetization these animals have not developed a severe grade of acid intoxication, and in several of the animals at the end of the experiment the alkali reserve of the blood was in excess of what it was at the end of the uranium intoxication and before an anesthetic was administered. Animals of this protected group have remained diuretic throughout the experiment and have shown an active diuresis from pituitrin, theobromine, and solutions of urea and sodium chloride. The kidneys of such animals

have shown histologically a normal vascular tissue, a convoluted tubule epithelium which gave the appearance of being hyperactive, and only occasionally were tubules encountered which showed signs of an early epithelial degeneration.

The nephropathic animals of the series in which a solution of sodium carbonate failed to afford any protection against Gréhant's anesthetic were animals over 1½ years old in which the uranium intoxication had resulted in a severer grade of acid intoxication than in the younger animals. When these older animals were given intravenously the carbonate solution and were anesthetized, it was found impossible to increase the alkali reserve of the blood to the same extent as was possible in the younger animals. Furthermore, the alkaline solution during the period of anesthesia is rapidly used up so that by the termination of these experiments the animals may have an alkali reserve of the blood which may be even lower than was the alkali reserve before the use of the carbonate. These animals have remained completely anuric throughout the experiments and have shown no diuretic effect from those diuretics which in the animals that were successfully protected by the carbonate induced free diuresis. The kidneys of these anuric animals show no degenerative changes in the glomerular vessels. The capillaries are not distended with blood as has been the case with the diuretic group. The epithelium of the convoluted tubules is acutely swollen. The swelling has frequently taken place to such an extent that the lumen of the tubules has become obliterated.

The nephropathic animals of the series which served as control animals and which were given a solution of sodium chloride equimolecular with the carbonate solution, following Gréhant's anesthetic became completely anuric. The sodium chloride solution furnished no protection against the anesthetic. The animals of all ages became anuric and unresponsive to the diuretic substances which have been used during this study. With the establishment of a state of anuria in these control animals the hydrogen ion content of the blood has increased, the alkali reserve of the blood has been rapidly depleted, and associated with this change the carbon dioxide tension has been reduced. The rapidity with which these changes develop

and the degree of acid intoxication which is induced is more marked in these animals than in any of the other series.

The kidneys of the control animals show the severest grade of degeneration of any of the nephropathic animals. The epithelium of the convoluted tubules is not only severely swollen but the cells frequently show necrosis. The loops of Henle contain more stainable fat than has been demonstrated in the kidneys of the carbonate animals.

CONCLUSIONS.

1. The toxicity of Gréhant's anesthetic for the nephropathic kidney is associated with the ability of the anesthetic to induce an acid intoxication. The severity of the acid intoxication increases with the age of the animal.

2. The efficiency of a solution of sodium carbonate to protect the kidney against the toxic effect of Gréhant's anesthetic depends upon its ability to prevent the development of an acid intoxication. The efficiency of a solution of sodium carbonate to furnish such a protection decreases as the age of the animal increases.

3. The inability of a solution of sodium carbonate to protect the kidney against Gréhant's anesthetic has been characterized by the development of an acute swelling of the renal epithelium which is later followed by necrosis. These animals develop an acute anuria and are unresponsive to diuretics.

4. The ability of a solution of sodium carbonate to protect the kidney against Gréhant's anesthetic has been associated with the histological preservation of the renal epithelium. These animals remain diuretic and responsive to various diuretic substances.

5. The inference should not be drawn from the observations which have been made that the epithelial damage is the sole cause for the anuria and the lack of response of some of the animals to diuretic substances. It is difficult to conceive that such gross changes in the volume of the renal epithelium could occur without seriously affecting the functional response of the vascular mechanism of the kidney.

EXPLANATION OF PLATES.

PLATE 3.

FIG. 1. Camera lucida drawing, Zeiss oc. 3, obj. 6. The figure is from the kidney of the carbonate animal of Experiment 3, Table I. The glomerular vessels show no degeneration and the capillary loops are well filled with blood. The epithelium of the convoluted tubules, *a*, is shrunken; the lumen of the tubules is prominent. The nuclei are large and hyperchromatic. At *b* the epithelium shows an early swelling. The animal was successfully protected against Gréhant's anesthetic by the carbonate solution, and was freely diuretic to both pituitrin and a 0.9 per cent solution of urea.

PLATE 4.

FIG. 2. Camera lucida drawing, Zeiss oc. 3, obj. 6. The figure is from the kidney of the carbonate animal of Experiment 5, Table I. The pathology of the kidney is in general similar to that shown in Fig. 1. The glomerular vessels fill the subcapsular space and the capillary loops are well filled with blood. The epithelium of the convoluted tubules, *a*, is shrunken; the nuclei of the cells are large and hyperchromatic. At *b* these cells show a beginning swelling. The animal was successfully protected against the anesthetic by the alkaline solution; it had a marked diuresis from both theobromine and a 5 per cent solution of sodium chloride.

PLATE 5.

FIG. 3. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of the carbonate animal of Experiment 11, Table I. The glomerulus is partially compressed by the swelling of the tubular epithelium. The capillaries are not distended with blood. The epithelium of the convoluted tubules, *a*, is severely swollen. The epithelium of the junctional tubules, *b*, fails to show the swelling. The animal was not protected against Gréhant's anesthetic by the carbonate solution. Early in the experiment it became anuric and showed no diuretic effect from theobromine or from a 5 per cent solution of sodium chloride.

PLATE 6.

FIG. 4. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of the control animal of Experiment 12, Table I. The animal received 25 cc. per kilo of a solution of sodium chloride equimolecular with a 3 per cent solution of sodium carbonate. The glomerular capillaries are not filled with blood. The epithelium of the convoluted tubules at *a* shows a severe grade of swelling. At *b* the cells have become necrotic. The junctional tubules at *c*

have an epithelium which is not swollen and in which the nuclei stain normally. During the development of a state of anesthesia the animal became anuric and remained anuric throughout the experiment No diuretic effect was obtained from either theobromine or a 5 per cent solution of sodium chloride.

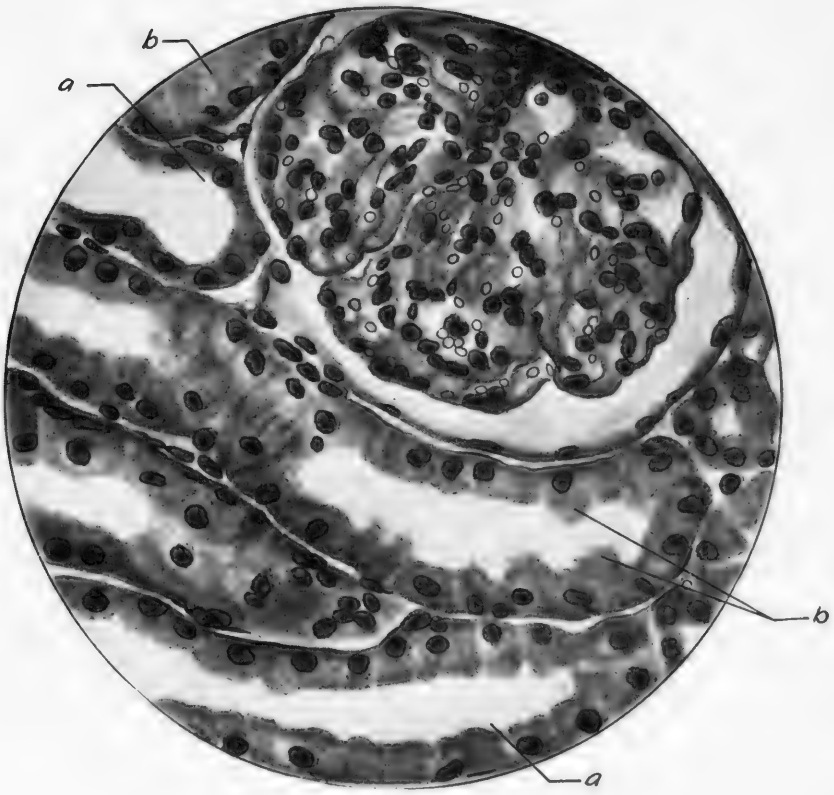


FIG. 1.

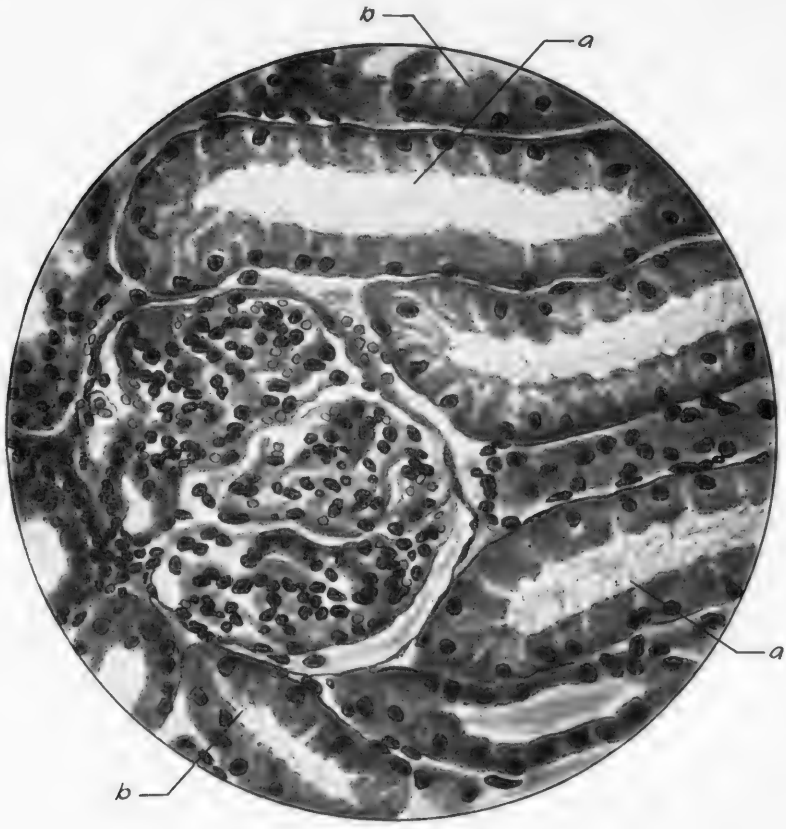


FIG. 2.

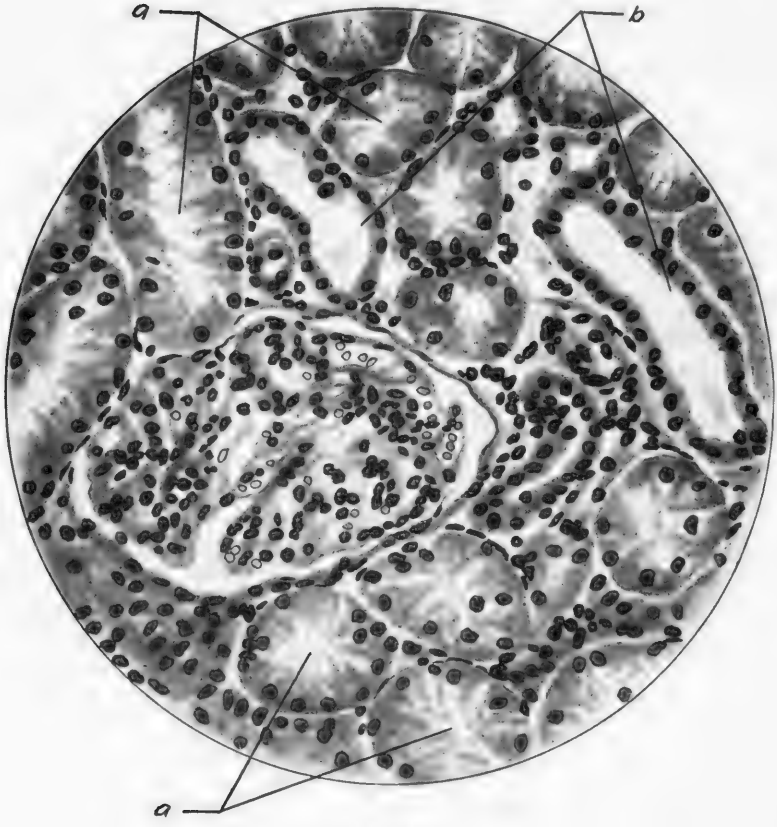


FIG. 3.

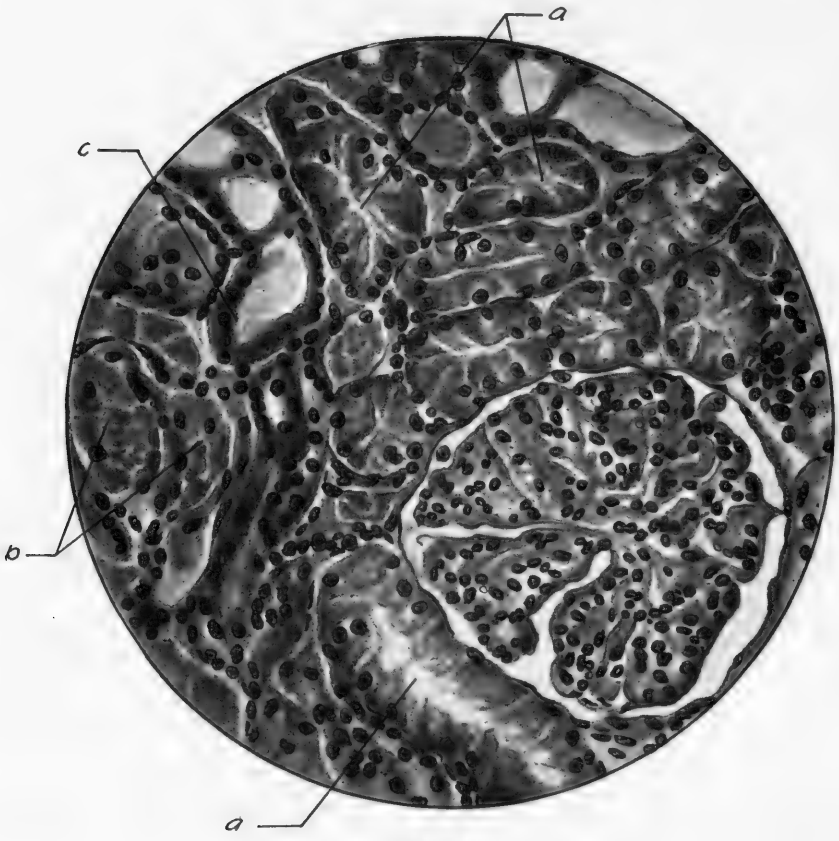


FIG. 4.

(MacNider: Toxicity of uranium. II.)

CULTIVATION OF SPIROCHÆTA OBERMEIERI.

By HARRY PLOTZ, M.D.

(*From the Pathological Laboratory of Mount Sinai Hospital, New York.*)

Plate 7.

(Received for publication, February 24, 1917.)

Up to the present *Spirochæta obermeieri* has not been grown from the blood of persons suffering from relapsing fever, nor has a spirochete of any species, as far as I have been able to discover, been cultivated directly from the blood of human beings. In this communication I wish to report the successful cultivation of *Spirochæta obermeieri* directly from the blood of patients suffering from European relapsing fever.

The studies were carried on in Serbia in the winter of 1915. The successful cultures, five in all, were made from cases clinically typical of European relapsing fever occurring in civilians and soldiers then residing in Macedonia.

Noguchi,[1] following his successful experiments on the cultivation of other spirochetes, succeeded in growing *Spirochæta obermeieri* from mice infected with relapsing fever. This spirochete had previously been kept alive by continued passage from animal to animal for a considerable length of time. The question arose whether spirochetes thus propagated for a long time become more amenable to artificial cultivation.

The method employed is the same as that used by Noguchi.[1] All cultures were taken during the febrile period of the disease. After careful disinfection of the skin with alcohol and iodine a vein in the antecubital space is punctured and 10 to 15 cc. of blood are withdrawn. A 15 cc. syringe with a thick bored needle is employed. It is important to withdraw the blood slowly, because rapid withdrawal may so injure the organisms that they will not grow. The needle is then removed from the syringe and the blood is slowly introduced into an Erlenmeyer flask containing 10 cc. of a 1.5 per cent sodium citrate solution in 0.85 per cent sodium chloride. The flask is gently agitated and the contents are then ready for culture. Culture tubes measuring 1.5 by 20 cm. are used.

[1] Noguchi, H., *J. Exp. Med.*, 1912, xvi, 199.

Into each tube is placed a piece of fresh sterile rabbit kidney tissue. The kidneys are removed aseptically and divided into about four pieces. About 5 cc. of the blood and sodium citrate mixture are added to each of a series of tubes. To this are added 15 cc. of sterile ascitic fluid. The ascitic fluid should be clear, free of bile and blood pigment, and should have a specific gravity of about 1.020. Noguchi has shown that an ascitic fluid which forms a fibrin filament when it comes in contact with the blood is best; no preservative should be added to the ascitic fluid and it should not be passed through a Berkefeld filter. Before use the fluid is tested out aerobically and anaerobically. Half the tubes are layered with sterile liquid paraffin. The tubes are incubated at 37°C.

The cultures were observed over a period of 2 weeks, but no definite appearance of growth was noted in the medium. Occasionally a slight clouding at the lower part of the ascitic fluid appeared. The tubes not layered with liquid paraffin showed the best growth.

Although only one or two spirochetes could be found in films made before culture (Fig. 1), a distinct increase could be noted after 48 hours' cultivation. In this series the maximum growth appeared on the 5th day and then gradually decreased. Preparations made from the junction of ascitic fluid and blood revealed masses and clumps of spirochetes. Preparations made from the upper part of the medium showed clumps and masses of spirochetes, but not so many as from the lower part (Figs. 2 and 3).

In young cultures the spirochetes appear shorter and thinner, but in older cultures they gradually become thicker and longer. The most striking appearance in young cultures is the spirochetal node which appears in practically every organism. These nodes are small thickenings which occur in the body of the spirochetes. From one to four may occur in the same organism. As the cultures become older these nodes become less numerous. I believe that these nodes probably represent the points where longitudinal division occurs.

Transplants are made about the 5th day by withdrawing 0.5 cc. of ascitic fluid from the lower part of the medium, without blood. This is inoculated into a similar tube as described above, except that 2 cc. of defibrinated human blood are added to each tube. The presence of hemoglobin aids the subsequent growth. In this manner *Spirochæta obermeieri* have been transplanted for five generations in two cultures.

EXPLANATION OF PLATE 7.[2]

FIG. 1. *Spirochæta obermeieri.* Blood film.
FIG. 2. Preparation from the upper part of the culture.
FIG. 3. Preparation from the lower part of the culture.

---

[2] The microphotographs were kindly made by Professor Julius Rothberger of the Sero-Therapeutic Institute, Vienna.

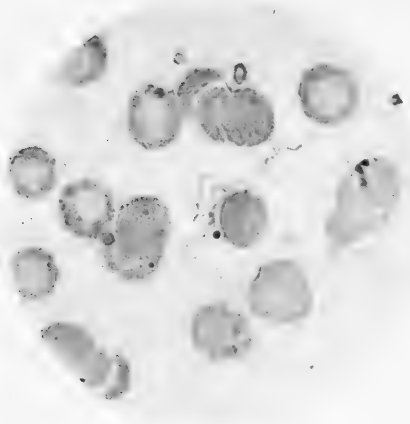


FIG. 1.

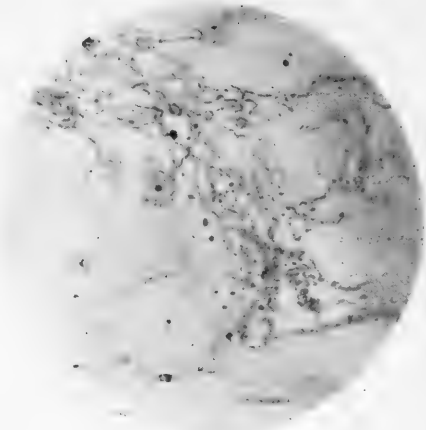


FIG. 2.

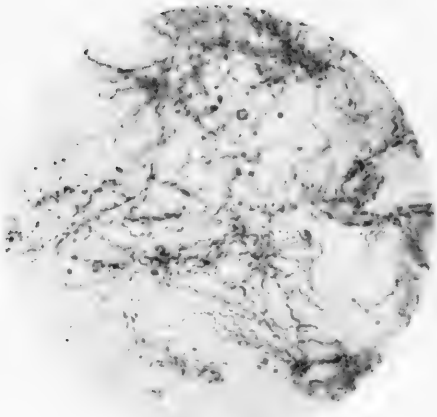


FIG. 3.

(Plotz: Cultivation of *Spirochæta obermeieri*.)

A VALVE TO REGULATE THE DELIVERY OF AIR AND ETHER VAPOR IN ANY PROPORTION.

By FREDERICK L. GATES, M.D.

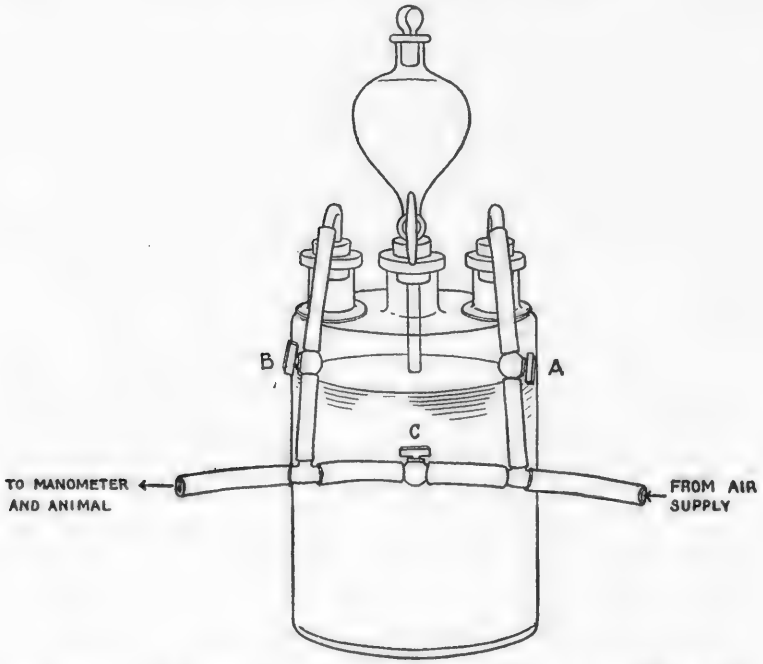
(*From the Department of Physiology and Pharmacology of The Rockefeller Institute for Medical Research.*)

(Received for publication, March 5, 1917.)

Since the introduction of the Meltzer-Auer method of intratracheal insufflation for artificial respiration and anesthesia in 1909, this method of delivering air and ether vapor under positive pressure at the bifurcation of the trachea has been widely adopted in laboratories of research and in the surgical clinic. The close regulation of air pressure and ether vapor tension, the constancy of delivery independent of the respiratory movements of the anesthetized subject, and above all, the safety and surety of the method in supporting life in spite of respiratory failure, combined with smoothness of anesthesia and freedom from danger of aspiration pneumonia, make it the most satisfactory method for human as well as laboratory surgery.

An extensive literature has grown up in this country and abroad which by its character attests the success of the method in principle and practice. Most of the authors agree on the correctness of the principles involved; most of them have some contribution to make in the way of new apparatus for the simplification and perfection of the technique or the mechanics of the method. Many types of respiration machines have been described, their variety proving the wide flexibility of the method and its adaptability to individual preference and need.

Dr. Meltzer has always insisted upon the utmost simplicity in apparatus, and in his laboratory a consistent attempt has been made to reduce the mechanics of intratracheal insufflation to the lowest terms consonant with efficiency. In this way mistakes and complications may be avoided and the mind of the operator freed from constant supervision of the respiratory apparatus and the anesthesia.



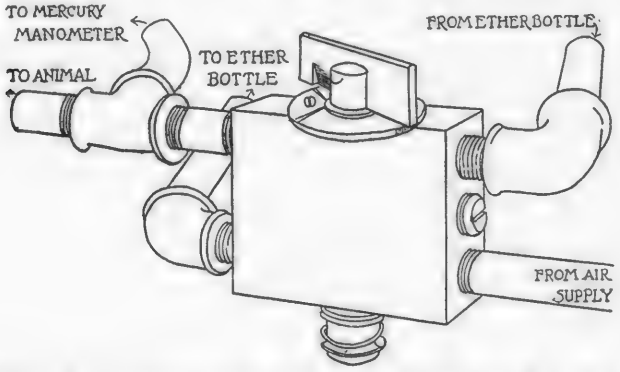
TEXT-FIG. 1. Woulfe bottle with the usual arrangement of tubing and stopcocks for air and ether vapor control. One-fourth the actual size.

Method of Obtaining Ether Vapor.

The simplest and most reliable method of obtaining ether vapor for insufflation anesthesia is the passage of the part of the air stream over the surface of ether in a Woulfe bottle (Text-fig. 1). The level of the ether is maintained by an auxiliary supply in a separating funnel. This is the method long in use in Dr. Meltzer's laboratory and has been adopted, with modifications, in several of the respiration machines intended for use in the clinic. If the ether surface is ample, it is not necessary to bubble air through the ether, or to warm the ether to promote evaporation, both of which are questionable procedures not unattended with danger.

Heretofore the diversion of a portion of the air stream over the

ether surface has been accomplished by means of stop-cocks in the tubing or some equivalent arrangement (Text-fig. 1). Three cocks are necessary for proper regulation of the ether supply. A and B control the air current in the ether circuit, while C regulates the passage of air unmixed with ether vapor. This arrangement has the disadvantage that any desired change in the proportion of ether vapor requires the manipulation of all three cocks to maintain the stream at its former volume and pressure, and even then it is practically impossible to determine the proportion of vapor-laden air obtained. It may be emphasized further that it is essential to have cocks A and B open to the same degree, lest an excess of ether vapor



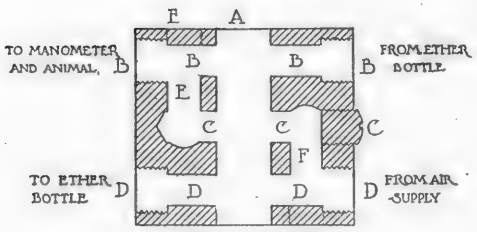
TEXT-FIG. 2. Constant volume valve, with fittings for the ether bottle and manometer. One-half the actual size.

be drawn into the tubing by the passage of air through C, or enter the system during the interruption of the air current if only one of the passages to the Woulfe bottle is subject to regulation.

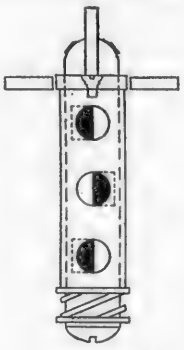
Description of the Valve.

The constant volume valve here described (Text-fig. 2) is in essence a convenient means of regulating all three stop-cocks synchronously, so that as the air stream over the ether is increased, the stream through C is reduced in exact proportion, and the sum of the volumes passing through the two limbs of the divided circuit remains constant. It consists of three channels corresponding to A, B, and C in Text-

fig. 1, but all contained in a single block, and all regulated synchronously by a single cock key through which the three channels are drilled. In the block (Text-fig. 3), D and B are the passages to and from the ether bottle, and C is the direct air passage in connection with D, F and E, B. As the passages B and D are closed by turning the cock key A, passage C must open in exact proportion so that the sum of the openings D (ether) and C (air) shall always equal a single opening of the same cross-section. This is accomplished by drilling the key so that the left edge of the holes corresponding to B and D are in line with the right edge of the hole corresponding to



TEXT-FIG. 3.



TEXT-FIG. 4.

TEXT-FIG. 3. Longitudinal median section of the brass block, drilled and plugged. One-half the actual size.

TEXT-FIG. 4. Cross-section of the block near the key showing the arrangement of the square ports in the key in relation to the passages for air and ether vapor. One-half the actual size.

C. Text-fig. 4 shows a cross-section of the block close to the key, which is set for half ether, half air. Moreover, the holes in the key must be square, cutting off segments with the circular holes in the block, the sum of which equals the cross-section of a single channel.

The dial plate over which the handle of the cock key turns is calibrated to show the proportion of the air stream which is sent over the ether in the Woulfe bottle. It should be pointed out that this is not an absolute index of the tension of ether vapor obtained, as that is further dependent upon the rate of evaporation of the ether

and the rate of flow of the air stream. We have not found it necessary or desirable in ordinary anesthesias to determine the vapor tension of the ether with accuracy. Many complicating factors come into account which make a record of ether tension, either in millimeters of mercury or in percentage of ether vapor, merely an abstract number, useful only for the reestablishment of similar conditions at some later time if desired. This can be done with sufficient accuracy for ordinary purposes by resetting the key handle on the dial scale to the point previously determined. It should be emphasized that the condition of the patient is always the criterion of the amount of ether to be given. The anesthesia is to be regulated by observation of the patient, not by the ether vapor tension on a scale. Even in the laboratory, where there is less at stake than in the clinic, we find that different animals of the same species require different amounts of ether to produce safe anesthesia under apparently similar conditions.

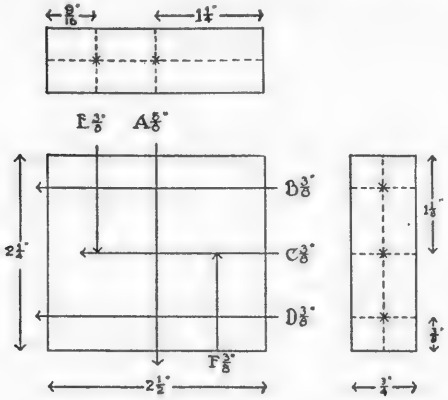
The valve described above has proved a simple and reliable means of regulating the ether vapor tension. Lately we have been employing this method of ether regulation for anesthesia in small animals, cats, rabbits, guinea pigs, rats, and mice, directing an ether-laden air stream to an ordinary cone, as used in the open drop method. The great advantages of intratracheal insufflation are not obtained, but the superiority over the open drop method of an unvarying supply of dry ether vapor, subject to close and instant regulation, is apparent. For one thing, the time of an assistant is saved.

Directions for Constructing the Valve.

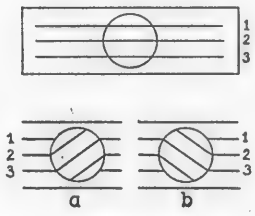
Since the valve can easily be turned out by a good technician or mechanic, working directions for its construction are appended.

The block is of brass, $2\frac{1}{2}$ by $2\frac{1}{4}$ by $\frac{3}{4}$ inches. Text-fig. 5 shows the centers and directions for drilling the channels. Hole A, $\frac{5}{8}$ inch in diameter, is for the key and should be drilled first, since it must be straight and true. Then B, C, and D, $\frac{3}{8}$ inch in diameter, are drilled in turn, B and D through the block, C to within $\frac{3}{8}$ inch of the opposite end. E and F, $\frac{3}{8}$ inch in diameter, join B to C at one end of the block and D to C at the other. B and D are drilled out at each end and tapped for $\frac{1}{4}$ inch pipe fittings. After the proper holes are

drilled through the key, C, E, and F are tapped out and plugs screwed in as far as F, B, and D respectively. The end of the plug in C may be cut as a screw head and used to regulate the size of F if the resistance of the passages to the ether bottle is found to be higher than that of the direct air passage C. The other plugs may be cut off flush with the block. A median longitudinal section of the block as drilled, tapped, and plugged is shown in Text-fig. 3.



TEXT-FIG. 5.



TEXT-FIG. 6.

TEXT-FIG. 5. Dimension drawing of the block as laid out for drilling. One-half the actual size.

TEXT-FIG 6. Bottom of the block with lines for setting the key. a, position of the key when C is drilled through it. b, position of the key when B and D are drilled. One-half the actual size.

The valve key, to fit properly, should be turned down to $\frac{5}{8}$ inch from larger stock and ground in with a very little emery and oil. A heavy grease used for lubrication will obviate any slight leak. The key is $3\frac{1}{4}$ inches long. The valve handle, of strip brass 2 by $\frac{3}{4}$ by $\frac{1}{8}$ inches, is riveted in a slot in the key, the other end of which projects $\frac{3}{8}$ inch beyond the bottom of the block and is fitted with washers and a spiral spring of moderate tension held in place by a screw or cotter-pin.

To drill the key properly, set the handle parallel with the long way of the block and draw three lines on the bottom of the block and key

as shown in Text-fig. 6. Line 2 is median, and lines 1 and 3 are parallel and $\frac{3}{16}$ inch from 2. Turn the key until 2 on the key intersects 3 and 1 on the block (Text-fig. 6a). Drill C through the key. Then turn the key back until 2 on the key intersects 1 and 3 on the block (Text-fig. 6b), or, better, until the exact point is reached at which air will no longer pass through C. Then drill B and D through the key. This is the position of the key for full ether. Turning the key back to the first position, it will be discovered that C (air) is fully open and B and D are just closed. Intermediate positions of the key handle will give various mixtures of ether vapor and air. Square up the holes in the key with a small square file. A dial plate made of a large brass washer (Text-fig. 2) may be attached to the block by machine screws and calibrated mathematically or by test to show the proportion of ether-laden air in tenths of the total volume. As described, the valve is tapped for $\frac{1}{4}$ inch pipe fittings as follows:

D, short pipe for tubing from the source of the air supply.
B and D′, close nipple, elbow, short pipe for tubing to the ether bottle.
B′, short nipple, tee, short pipes for manometer and for tubing to the animal.

The test of the valve's efficiency is that under working conditions it shall maintain a constant pressure in the mercury manometer when the valve handle is turned slowly between full ether and full air. If there is a rise in the pressure in the direction of full air, the resistance of the air circuit may be raised to that of the ether circuit by screwing in the plug C until it begins to cut off passage F.

SUMMARY.

A valve is described for the control of ether vapor for anesthesia which regulates the mixture of ether vapor from a Woulfe bottle with air in any proportion, without changing the volume or the pressure at which the mixture is delivered. The regulation of the air stream both to and from the ether bottle controls the mixture accurately and is an essential feature of the valve. Except for experimental purposes, it is not considered profitable to determine on an arbitrary scale the tension of the ether vapor obtained, because the depth of the anesthesia should always be judged by the condition of the subject.

The valve was devised for the Meltzer-Auer method of intratracheal insufflation, but it is adapted for use wherever a constant mixture of air and ether vapor is desired. Several respiration machines for supplying and interrupting the air stream, using this constant volume valve for the regulation of the ether supply, have been in use in Dr. Meltzer's laboratory for periods up to 2 years, and the simplicity and efficiency of the valve have been thoroughly tested. A description of the complete machine will be published later.

THE RELATION OF MOSQUITOES AND FLIES TO THE EPIDEMIOLOGY OF ACUTE POLIOMYELITIS.

By HIDEYO NOGUCHI, M.D., and ROKUSABURO KUDO, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, March 15, 1917.)

Of several hypotheses concerning the mode of conveyance of poliomyelitis, that of direct contact, supported by Flexner, appears to be the only one which stands on a substantial experimental basis.[1,2] Although in all epidemics instances have been reported in which more than two members of a family were victims of the disease, as a rule only one out of several children in a family is attacked. This peculiar feature of the epidemiology of poliomyelitis seemed to support the theory that the disease may be inoculated by the bite of an infected insect rather than transmitted directly from one individual to another.

Typical examples of insect transmission of disease are found in yellow fever, malaria, spirochetoses, trypanosomiases, and other tropical fevers. Rosenau and Brues[3] in 1912 announced their success in transmitting poliomyelitis from an infected to a normal *Macacus* monkey by means of the bites of stable-flies (*Stomoxys calcitrans*). Their experiment consisted in allowing many thousands of the flies to feed first on an infected monkey and immediately afterwards on a normal monkey. The transmission was mechanical, but not in the sense of an intermediary host. Anderson and Frost[4] confirmed Rosenau's finding in a

[1] Flexner, S., The mode of infection and etiology of epidemic poliomyelitis, *Am. J. Dis. Child.*, 1915, ix, 353.

[2] Flexner, The nature, manner of conveyance and means of prevention of infantile paralysis, *J. Am. Med. Assn.*, 1916, lxvii, 279.

[3] Rosenau, M. J., and Brues, C. T., Some experimental observations upon monkeys concerning the transmission of poliomyelitis through the agency of *Stomoxys calcitrans*, *Tr. XVth Internat. Cong. Hyg. and Demography*, Washington, 1913, i, 616.

[4] Anderson, J. F., and Frost, W. H., Transmission of poliomyelitis by means of the stable fly (*Stomoxys calcitrans*), *Pub. Health Rep., U. S. Mar. Hosp. Serv.*, 1912, xxvii, 332.

limited number of instances, and then later failed to obtain further positive results. Howard and Clark[5] were unable to transmit the infection from an infected to a normal monkey by the bites of *Stomoxys calcitrans*. The peculiarity of conveyance of the infection is far from being solved by the theory of transmission by these blood-sucking flies.

The most plausible hypothesis so far advanced seems to be that offered by Amoss and Taylor,[6] who, having experimentally demonstrated the existence of a protective principle in the secretion of the nasal mucous membranes of normal individuals, consider that the incidence of infection may to a considerable extent depend upon the condition of these mucous membranes; that is, as long as they remain intact, the virus will be destroyed before it can become established in the individual. The disturbance of this natural protective mechanism exposes to infection, but not the presence or absence of the virus alone. They were able to show that the protective substance in question was more generally and abundantly present in adults than in young children.

The sudden disappearance of poliomyelitic cases with the return of cold weather is often used as an argument in favor of insect transmission of the disease. Flies and mosquitoes have constantly been under suspicion. While the elimination of these insects from our households is desirable as a matter of routine cleanliness, yet without a systematic experimental investigation they should not be accepted as a factor in the epidemiology of poliomyelitis. Indeed, it would be unwise if, through a misapprehension of the facts, the attention of health officers and laity alike were to be diverted from the real source of danger.

Scope and Mode of Experiments.

The question whether a given variety of insect plays a part in the spread of poliomyelitis is within reach of exact determination, since these insects are amenable to artificial propagation through many generations. During the past several months, we have been able to collect and propagate in tanks in the laboratories a quantity of *Culex pipiens* from sewer waters near Jersey City[7] and in

[5] Howard, C. W., and Clark, P. F., Experiments on insect transmission of the virus of poliomyelitis, *J. Exp. Med.*, 1912, xvi, 850.

[6] Amoss, H. L., and Taylor, E., Neutralization of the virus of poliomyelitis by nasal washings, *J. Exp. Med.*, 1917, xxv, 507.

[7] We are greatly indebted to Dr. Headlee, chief entomologist of the New Jersey Agricultural Experiment Station, at New Brunswick, and to Dr. Chidester

Queens County, Long Island. It was our intention to study several well known genera of mosquitoes at the same time, but owing to the lateness of the season, none of the *Aedes* or *Anopheles* species were available. The only variety which we were able to obtain was *Culex pipiens*. Experiments were also performed to determine whether the common house-fly or the bluebottle fly is able to take up the poliomyelitic virus and make it increase within its body.

There are at least two totally different ways of attacking the problem. The first method is to feed young growing larvæ with the active poliomyelitic virus by putting it in the polluted water in which they are being grown. When the adult mosquitoes have hatched out, they may be allowed to feed on a normal *Macacus* monkey. The second procedure is that ordinarily practised by investigators and consists in allowing the mosquitoes to feed first on an infected animal and then, within a certain length of time, on a normal animal.

The experiments with non-biting flies had to be carried out by still another method. They were allowed to feed on the nervous tissues containing an enormous amount of the poliomyelitic virus until they pupated. Part of the pupæ were allowed to hatch. Both the pupæ and the imagos were then crushed together and the emulsion was filtered through a Berkefeld filter. The clear filtrate was introduced intracerebrally and subsequent events were observed.

Experiments with Mosquitoes Hatched in Polluted Water Experimentally Contaminated with the Virus of Poliomyelitis.

To twelve jars, covered with a wire net cage and containing mosquito larvæ of various ages in ordinary stagnant water, were added varying amounts of the brain or cord emulsions or their filtrates derived from two monkeys that died of experimental poliomyelitis. The virus was introduced every 24 hours for a period of 10 days. The temperature of the laboratory was kept at about 70°F. and the

of Rutgers College, New Brunswick, for their assistance and instruction as to the propagation of mosquitoes in the laboratory. We wish to express our gratitude also to Mr. Winchell of Rutgers College, who assisted us in collecting the specimens in the field.

relative humidity at about 40 per cent. Numbers of adult mosquitoes, both male and female, hatched out from the jars every day. They were carefully collected and used for transmission experiments on normal monkeys. The rate of hatching from day to day is shown in Table I.

TABLE I.

Date.	Female.	Male.
1916		
Oct. 31	21	20
Nov. 1	12	9
" 2	11	2
" 3	17	8
" 5	10	12
" 6	15	8
" 7	30	18
" 8	25	9
" 9	13	18
Total.....	154	104

The first transmission experiment was performed with mosquitoes on two young *Macacus rhesus* monkeys. As the mosquitoes attack the monkeys only during the night, the experiment was begun at dusk and discontinued the next morning. The results were as follows: Of 150 females and 60 males put into the cage, 114 females were fully engorged with the blood, 23 females were apparently not engorged, and 52 males were still living. Some of the mosquitoes had been killed by the monkeys.

Close observation was maintained on the monkeys for 21 days, but no sign of poliomyelitis developed nor has any been noticed up to the present time.

Some of the engorged females sooner or later laid eggs and perished, while some died without oviposition. A number of female mosquitoes hatched out of the egg boats and also from the virus-containing water were kept alive for about 3 weeks and then allowed to bite two young *Macacus* monkeys. There were about 40 well engorged specimens when examined the following morning. The monkeys showed no symptoms of poliomyelitis.

These experiments demonstrate that the mosquitoes raised in water containing the virus of poliomyelitis in large quantities cannot transmit the infection by their bites to normal *Macacus* monkeys. There is no indication that the virus enters and multiplies in the body of mosquitoes, even if 3 weeks are allowed to elapse between the time of hatching and that of the biting experiment. The offspring of these females are not capable of transmitting the disease to the monkey.

Experiments to Determine Whether the Females Well Engorged with the Blood of Infected Monkeys Will Ever Be Capable of Transmitting the Infection by Their Bites to Normal Macacus Monkeys.

In order to decide this point, eight groups of mosquitoes, some obtained from the vicinity of Jersey City, and some from Queens County, Long Island, were allowed to feed on several *Macacus* monkeys inoculated for this purpose. To insure ourselves of including different stages of the infection, feedings were made every 24 hours after the inoculation of the virus into the monkeys and were extended into the paralytic stage, which of course varied in different animals from 6 to 9 days, according to the activity of the virus and the quantity injected. The protocols of these feeding experiments show the time which elapsed between the feedings and the transmission experiments. The term "feeding mosquitoes" is used merely to denote that these mosquitoes have sucked blood from an infected monkey.

Four out of the eight groups were tested on November 20; that is, 1 to 2 weeks after the time of feeding. The remaining four groups were allowed to bite after having been kept from 2 to 3 weeks. It must be mentioned that the engorged females do not survive for any length of time after oviposition, and comparatively few specimens remained alive in the second series of groups.

For the purpose of transmission two young *Macacus* monkeys were employed for each of the two experiments (Groups 1 to 4 and 5 to 8). We also inoculated 2 cc. of sterile normal horse serum into the intrathecal space in order to derange the protective mechanism which the meninges provide against the penetration of the virus

into the central nervous system in experimental poliomyelitis in monkeys. It may be recalled that Flexner and Amoss[8] demonstrated that a fraction of the virus, intravenously introduced, will readily localize in the central nervous system when the meninges are previously disturbed by the inoculation of various substances, although the monkey may bear a multiple of such doses with impunity if there has been no previous meningeal injury.

Group 1. Feeding Mosquitoes.—Nov. 13, 1916. Adult mosquitoes hatched in the laboratory from the larvæ collected in Queens were allowed to feed on *Macacus rhesus* 1 on the 4th day of experimental poliomyelitis. 30 mosquitoes were well engorged when examined the next morning. Used for experiments on Nov. 20, or in 7 days.

Group 2. Feeding Mosquitoes.—Nov. 15, 1916. Another lot of adult mosquitoes hatched in the laboratory from the same material were fed on the same monkey (No. 1) on the 6th day of the disease. The animal showed partial paralysis of the limbs. 23 well engorged females were obtained. Used for experiments on Nov. 20, or in 5 days.

Group 3. Feeding Mosquitoes.—Nov. 16, 1916. Another lot of adults from a similar source were fed on the same monkey (No 1) on the 7th day of the disease. The animal was completely paralyzed. 17 engorged females were collected. Used for experiments on Nov. 20, or in 4 days.

Group 4. Feeding Mosquitoes.—Nov. 7, 1916. A lot of adults hatched in the laboratory from the larvæ from Queens were fed on *Macacus rhesus* 2 on the 9th day of the disease. 25 engorged females were obtained. Used for experiments on Nov. 20, or in 13 days.

Group 5. Feeding Mosquitoes.—Dec. 4, 1916. A lot of adult mosquitoes hatched in the laboratory from the larvæ secured from a Jersey City suburb were fed on two *Macacus rhesus* monkeys, Nos. 3 and 4, on the 7th day of experimental poliomyelitis. 13 engorged females were obtained. Used for experiments on Dec. 19, or in 15 days.

Group 6. Feeding Mosquitoes.—Dec. 1, 1916. A lot of adult mosquitoes hatched in the laboratory from the materials gathered in the neighborhood of Jersey City were fed on *Macacus rhesus* 5 on the 3rd day of experimental poliomyelitis. 34 engorged females were obtained. Used for experiments on Dec. 15, or in 14 days.

Group 7. Feeding Mosquitoes.—Nov. 24, 1916. A lot of adult mosquitoes hatched in the laboratory from the material from Queens were fed on *Macacus rhesus* 6 on the 2nd day of the disease. 45 engorged females were obtained. Used for experiments on Dec. 15, or in 21 days.

---

[8] Flexner, S., and Amoss, H. L., The relation of the meninges and choroid plexus to poliomyelitic infection, *J. Exp. Med.*, 1917, xxv, 525.

Group 8. Feeding Mosquitoes.—Nov. 25, 1916. A lot of adult mosquitoes hatched in the laboratory from the material from Queens were fed on *Macacus rhesus* 7 on the 5th day of the disease. The animal had not yet become paralyzed. 20 engorged females were collected. Used for experiments on Dec. 15, or in 20 days.

The results of the experiments were negative, in spite of the fact that many females (about fifty) attacked the monkeys and became fully engorged with the blood sucked from them.

As in the earlier series of experiments, many of these engorged females oviposited and gave rise to a new generation of larvæ. It was easy to propagate them in the laboratory for an indefinite period. These offspring were in turn allowed to bite a normal monkey, but so far no positive transmission of the infection by their bites has resulted.

Experiments with Non-Biting Flies Reared in the Laboratory with an Abundant Quantity of Poliomyelitic Virus.

Non-biting adult flies may mechanically carry the virus of poliomyelitis just as they do various pathogenic microorganisms.[9] But no experimental evidence has been adduced to prove whether the virus can penetrate into the interior of the fly larvæ when the latter are fed on the poliomyelitic material, or, if it does, whether it is capable of multiplication within the body cavity of the larvæ. The question here is not the same as that of blood-sucking flies or mosquitoes, which may be the intermediary hosts as well as the direct transmitters of the virus by their bites. But in the case of non-biting flies, though the virus may multiply in their bodies, its distribution must depend upon their well known habits of gathering about food or persons while they regurgitate, resuck, and excrete. In this way the question of the non-biting flies as intermediary hosts of the virus would be quite as important as that of the biting varieties.

In the present experiment, a number of young larvæ of house-flies (*Musca domestica*) and of bluebottle flies (*Calliphora vomitoria*) were fed with the brain tissue of a *Macacus rhesus*, which had died of ex-

[9] Flexner, S., and Clark, P. F., Contamination of the fly with poliomyelitis virus. Tenth note, *J. Am. Med. Assn.*, 1911, lvi, 1717.

perimental poliomyelitis with typical symptoms and pathological findings. The activity of the material used was established in a subsequent experiment.

Group 1.—Nov. 23, 1916. About 50 house-fly larvæ were put on several slices of the fresh poliomyelitic monkey brain (No. 1). The larvæ eagerly penetrated into the nervous tissue as if trying to shield themselves from the light. At room temperature the tissue underwent a putrefactive decomposition within 24 hours, so the larvæ had to be placed on a fresh lot of the virus-containing brain. When left in the decomposed mass of tissue, many larvæ died. After being fed on the poliomyelitic brain for about 6 days, the larvæ became pupæ. After pupation, they were left with the brain tissue for several days longer and then washed from the tissue and removed to the refrigerator.

Group 2.—Nov. 28, 1916. Another lot of house-fly larvæ were similarly fed with poliomyelitic brain emulsion (No. 1) for 6 days. Pupation took place on Dec. 4. The pupæ were washed and removed to the refrigerator on Dec. 9.

Group 3.—Nov. 23, 1916. 25 larvæ of bluebottle flies were fed on several slices of brain tissue from a *Macacus rhesus* which had died of poliomyelitis. The larvæ attacked the tissue vigorously and within 24 hours seemed to have consumed a great deal of it. Unlike the house-fly larvæ, they feed on dead flesh or meat during their larval stage, but it was necessary to put them on fresh material every 24 hours in order to protect them from the deadly effect of the decomposed nervous tissue. Pupation occurred on Dec. 7. After several days, the pupæ were washed and removed to the refrigerator.

For the purpose of obtaining adult flies from the infected pupæ, a dozen specimens from each lot were put in a Petri dish and left at 80° F. Imagos hatched within about 12 days. These and the pupæ were used for the experiment described below.

Preparation of Filtrate of the Infected Pupæ and Imagos of the House-Fly and the Bluebottle Fly.

70 house-fly pupæ, Groups 1 and 2. 12 " imagos, " 1 " 2.	Weight 1.36 gm.
15 bluebottle fly pupæ, Group 3. 6 " " imagos, " 3.	" 0.97 "

These were mixed together and thoroughly ground up in a mortar, 22 cc. of 0.9 per cent saline solution being added. The milky emulsion thus obtained was briefly centrifuged at a low speed to separate the coarse particles from the fluid. The latter was then passed through a sterile Berkefeld filter V, the result being a clear and sterile filtrate.

Animal Inoculation.—A *Macacus rhesus* was inoculated intracerebrally with 3 cc. of the above filtrate on Dec. 16, 1916. The result was negative, no symptoms suggestive of poliomyelitis having been observed.

The foregoing experiment indicates that it is improbable that the virus of poliomyelitis is taken up by fly larvæ and multiplies therein. Not only does the virus fail to multiply, but probably it is rapidly destroyed within the body of the insects. The notion that these non-biting flies may act as intermediary hosts or a virus reservoir is not justified by the evidence brought out in the present study.

SUMMARY.

1. *Culex pipiens* raised from the larval stage in water experimentally contaminated with an abundance of poliomyelitic virus were found to be incapable of causing the infection when allowed in large numbers to bite normal *Macacus* monkeys.

2. *Culex pipiens* which were fed on infected poloimyelitic monkeys during different stages of the disease were found to be incapable of transmitting the infection when allowed in large numbers to bite normal *Macacus* monkeys. A previous disturbance of the meninges by an injection of horse serum into the intrathecal space did not alter the result, which was negative.

3. The offspring of the mosquitoes which were either reared in the infected tanks or fed on infected monkeys were found to be entirely harmless when allowed to feed in large numbers on a normal monkey. There was no hereditary transmission of the virus from one generation to another.

4. No trace of the virus of poliomyelitis was demonstrable in the filtrate of an emulsion of adult flies and pupæ of the common housefly and bluebottle fly which were reared in the laboratory on slices, emulsion, or filtrate of monkey brain containing the poliomyelitic virus. The intracerebral injection of the filtrate produced no poliomyelitic infection in the normal monkey.

NEW ANAEROBIC METHODS.*

By WILSON G. SMILLIE, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

PLATE 8.

(Received for publication, March 12, 1917.)

The methods commonly used for the cultivation of anaerobic microorganisms are far from satisfactory. This applies to the partial anaerobes, such as the tetanus bacillus or *Bacillus botulinus*, but is particularly true of the absolute anaerobes, such as *Treponema pallidum* or the globoid bodies of poliomyelitis.

The vacuum jar described by Noguchi[1] in his method for the cultivation of *Treponema pallidum* has proved satisfactory in his hands, but requires a very strong desiccator, accurately ground, and a special type of vacuum pump. The American desiccators which I have obtained during the past 2 years, however, have either been so poorly ground that they would not hold a vacuum, or else they were so poorly made that they collapsed when subjected to vacuum.

After many trials, my difficulties were finally overcome through utilization of the suggestions of Laidlaw,[2] who used a catalyzer of oxygen and hydrogen in the preparation of anaerobic culture tubes. McIntosh and Fildes[3] developed the use of the catalyzer in making an anaerobic container, the "McIntosh bomb," but neither of these methods, useful as they are for the purposes for which they were devised, were suitable to our needs, and were therefore modified.

All the anaerobic methods to be described depend upon the catalytic action of platinized asbestos upon oxygen and hydrogen when they are brought into contact.

* This work was done under the tenure of a William O. Moseley Travelling Fellowship from Harvard University.

[1] Noguchi, H., *J. Exp. Med.*, 1911, xiv, 99.

[2] Laidlaw, P. P., *Brit. Med. J.*, 1915, i, 497.

[3] McIntosh, J., and Fildes, P., *Lancet*, 1916, i, 768.

Methods for Anaerobic Test-Tubes.

The simplest method is used in the cultivation upon agar slants of such relative anaerobes as the tetanus bacillus or *Bacillus botulinus* and is similar to the method described by Laidlaw. Platinized asbestos is first prepared in the usual way, or it may be purchased from any laboratory supply house. A small mass of the catalyzer is firmly fixed at the end of a platinum wire by coiling the wire about it. The other end of the wire is inserted into a short glass rod, and the rod is inserted into a No. 1 one-hole rubber stopper. The apparatus is wrapped in a package and autoclaved.

The water of condensation is removed from a plain agar slant, the tube inoculated, inverted, the cotton plug removed, and the tube filled with hydrogen by means of a sterile capillary pipette. The hydrogen may be obtained from a Kipp generator, or more satisfactorily from a hydrogen tank. It should be passed through a series of wash bottles containing silver nitrate, sulfuric acid, potassium permanganate, and lead acetate to remove all impurities.

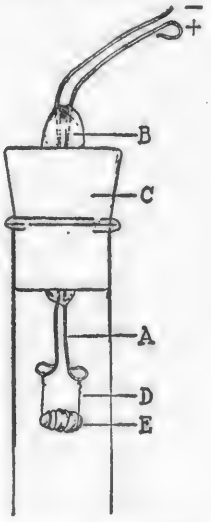
After allowing the hydrogen to fill the inverted inoculated test-tube, the platinized asbestos is heated for a moment in a free flame, the rubber stopper is inserted firmly into the inverted tube, and the end of the tube dipped into melted paraffin.

The catalyzer glows for a second or two as the hydrogen and oxygen are actively united, and the water formed is deposited upon the surface of the tube. The process is now complete, and the tube is ready for incubation.

This is essentially the method devised by Laidlaw and is very satisfactory for the growth of the usual anaerobes. The tetanus bacillus, for example, grows upon an agar slant in a thick, felt-like mass, in the profusion of its growth resembling a culture of *Bacillus subtilis*. It was necessary to devise a more strictly anaerobic method for our work, however, for Laidlaw's method could not always be relied upon to remove all traces of oxygen in the air, nor does it remove the oxygen from the surface of the media itself. The following method was devised to remove all the oxygen and has proved very satisfactory.

Two lengths of No. 22 nichrome wire, 6 cm. long, are separately

fused into a glass tube so that they are insulated (Text-fig. 1, A), and the glass tube, B, closed at each end, is passed through a one-hole rubber stopper, C. To the lower ends of the nichrome wire is attached a coil of fine (No. 31) nichrome wire, D, thus completing the circuit. In the coils of the fine wire is placed a small mass of platinized asbestos, E. The apparatus is placed in a package and autoclaved.



TEXT-FIG. 1. Sketch of anaerobic apparatus for the cultivation of absolute anaerobes in test-tubes.

A large test-tube, 20 by 1.5 cm. is used, to which 10 cc. of media are added, sterilized, and slanted. The water of condensation is removed and the tube inoculated. The tube is inverted, the cotton plug removed, and the tube filled with hydrogen by means of a sterile capillary pipette. The platinized asbestos mass, E, is heated for a moment in a free flame, and the rubber stopper, C, is then firmly inserted into the inverted tube and the end of the tube dipped in melted paraffin. The tube may now be placed in an upright position and sufficient electric current applied to the free ends of the wire to heat the fine nichrome wire wrapped about the platinized asbestos to a red heat. The catalyzer is thus heated, and the free

oxygen and hydrogen unite to form water. The tube is set aside for ½ to 1 hour, then the platinized asbestos is reheated in order to ignite any residual oxygen. The tube may now be incubated.

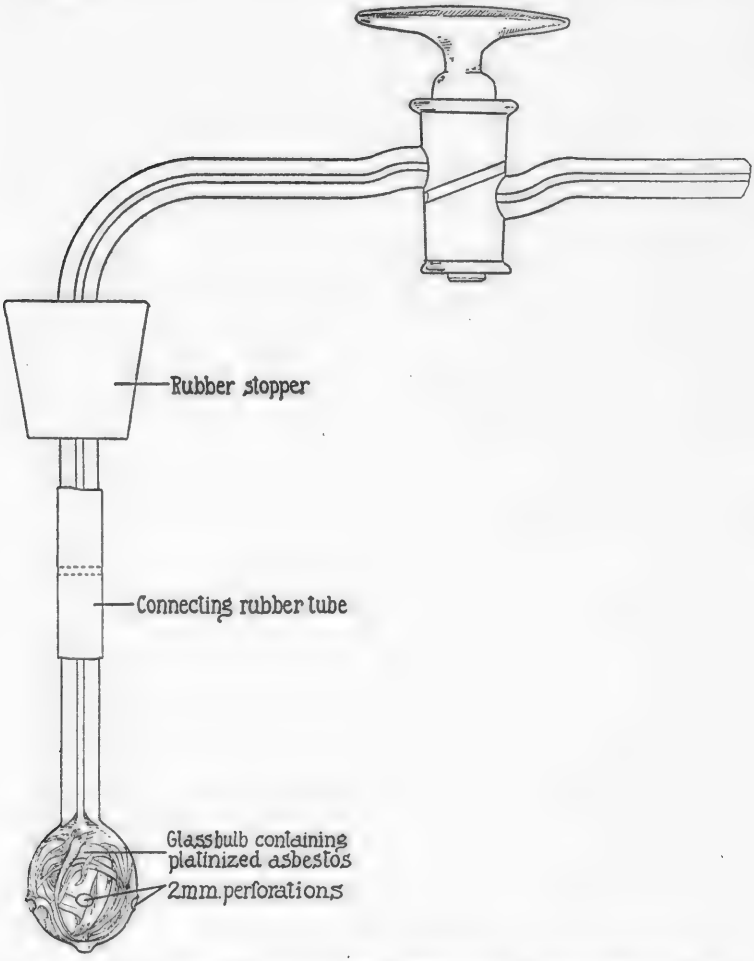
The method is very useful in growing all anaerobes, for the oxygen is always removed, whereas the Laidlaw method frequently fails. It is particularly useful for the cultivation of the stricter anaerobes. By this method the fusiform bacillus, for example, which is more strictly anaerobic than the tetanus bacillus, grows profusely in a thick, felt-like mass. By using this technique the organism of poliomyelitis grows upon ascitic agar slants, though much more slowly than other organisms; in fact, no growth is seen for 4 to 5 days, but at the end of 6 to 7 days, definite, tiny, raised, glistening colonies appear. These grow larger and larger, so that at the end of 12 to 14 days a definitely circumscribed, raised, opalescent colony is seen, some of these colonies even becoming as large as 1 mm. in diameter. In no instance was a growth of the globoid bodies of poliomyelitis obtained from the original material—brain and cord—but only from the fluid culture tubes of globoid bodies which had been growing in ascitic fluid media for several generations.

Method for an Anaerobic Jar.

The chief problem with which I was concerned, however, was the construction of an anaerobic jar, to be used as a container for a large number of tubes in the isolation of the organism of poliomyelitis from the infected brain and other tissues. All methods used for the cultivation of the globoid bodies failed in my hands until the jar described below was devised.

The jar used is an ordinary museum specimen jar (Fig. 1) about 30 cm. high and with an inside diameter of 12.5 cm. Two holes, 1.5 cm. in diameter, are ground in the cover, and into each hole is firmly inserted a No. 4 one-hole rubber stopper, carrying a ground glass "angle" stop-cock. To one of the stop-cocks is attached a rubber tube, at the end of which is a short piece of glass tube which reaches to the bottom of the jar. To the other stop-cock is attached, by a short rubber tube, a glass bulb, 2 cm. in diameter, which has been blown on the end of a capillary glass tube. The glass bulb is perforated with 5 to 6 holes, 2 mm. in diameter, and is filled with

platinized asbestos. The details of this apparatus are shown in Text-fig. 2.



TEXT-FIG. 2. Detail of the platinized asbestos bulb for the anaerobic jar.

Cultures are inoculated and placed in a glass tumbler, which is then placed in the jar, to which 100 cc. of a 10 per cent pyrogallic acid solution have been added.

The glass bulb containing the platinized asbestos is heated over the free flame for a few seconds, and the cover is then cemented on. A rubber ring, 0.5 cm. thick, is placed between the jar and cover, all surfaces are cemented with Major's glass cement, and the metal clamp is screwed down with thumb and forefinger. The stop-cock to which the glass bulb is connected is placed on the vacuum pump, and gentle suction is applied for 2 to 3 seconds in order to insure a good initial flow of hydrogen and thus ignite the platinized asbestos at once. The stop-cock is now closed and attached to the hydrogen apparatus, and the gas is allowed to enter. This should be done carefully at first in order that an excess of hydrogen does not enter at once; for the gas should be burned as rapidly as it enters the jar. The platinized asbestos will soon be seen to glow and from this time hydrogen and oxygen will slowly unite, and the water formed will be deposited on the sides of the jar. When all the oxygen has united with the hydrogen, the platinized asbestos will become cool, but the hydrogen will continue to enter the jar until all the space formerly occupied by oxygen is replaced by hydrogen. The result is a hydrogen-nitrogen jar under approximately atmospheric pressure. The whole process should take about 15 minutes.

In order to have an index of the completeness of anaerobiosis the second stop-cock is connected with a bottle of 20 per cent sodium hydrate, freshly washed with hydrogen. By means of slight suction through the first stop-cock, 25 cc. of the sodium hydrate solution are drawn into the jar. Both stop-cocks are now closed, the ends sealed with cement, and the jar is incubated.

If the jar is satisfactory, the mixture of sodium hydrate and pyrogallic acid will remain colorless indefinitely. This solution should not be relied upon to absorb any remaining traces of oxygen, but is simply an indicator of the presence of oxygen, and if it becomes discolored, there has been a mistake in technique, and the jar is unsatisfactory; therefore the cover should be removed, and the process repeated.

Method for Blake Bottles and Flasks.

The use of the platinized asbestos in a perforated glass bulb has been applied to mass cultures of anaerobes in flasks or Blake bottles.

The Blake bottle is useful when large amounts of an anaerobic organism, such as the tetanus bacillus, are desired, and is particularly useful when a differential anaerobic plate is desired as, for example, in the isolation of anaerobes from the pus of infected wounds.

A ground glass stop-cock of the usual type is inserted through a No. 3 one-hole rubber stopper. A heavy glass bulb, 1 cm. in diameter, is blown at the end of the stop-cock, and five or six small perforations are made in the bulb. The bulb is then filled with platinized asbestos, and the whole apparatus autoclaved.

The Blake bottle is inoculated, the platinized asbestos heated in a free flame, and the rubber stopper tightly inserted into the bottle. Slight vacuum is then produced in the bottle by gentle suction, in order to insure a good flow of hydrogen. The stop-cock is connected with the hydrogen generator and the gas allowed to enter. The catalyzer glows for a few minutes, then cools, and in 5 minutes the oxygen has been replaced by hydrogen, and the process is complete.

The use of the perforated bulb has not proved satisfactory in single test-tubes because of the small air space.

The methods are simple, rapid, clean, and efficient. They are not expensive, for only a small amount of platinized asbestos is needed, and the material may be used repeatedly without deterioration. It would at first appear that there might be some danger of explosion in the jar, but such is not the case. The hydrogen ignites as soon as it enters the jar so that there is never an excess of hydrogen in the container. As the hydrogen and oxygen unite to form water, a slight vacuum is formed and this vacuum insures a continuous gentle flow of hydrogen until all the oxygen has been replaced.

The precautions to be taken are as follows: (1) Allow the hydrogen to enter the jar slowly. (2) Be sure that the hydrogen is catalyzed as it enters, as evidenced by the glowing of the asbestos. After the glow has once appeared the remainder of the process will continue gently and completely. (3) Do not disconnect the apparatus while the process is taking place, for there is a slight vacuum in the jar which has only partially been replaced by hydrogen, and if air is allowed to rush in over the catalyzer, there is a possibility of a slight explosion.

The results that have been obtained by the use of these methods, particularly the anaerobic jar, will be published in a subsequent paper.

CONCLUSIONS.

1. Anaerobic methods have been devised which depend upon the catalytic action of platinized asbestos upon hydrogen and oxygen.

2. The methods may be utilized for the growth of anaerobes in test-tubes, upon Blake bottles, in flasks, and in a large container.

3. Because oxygen is so completely removed, the methods are of great value in the successful cultivation of absolute anaerobes.

EXPLANATION OF PLATE 8.

FIG. 1. Anaerobic jar with platinized asbestos bulb.

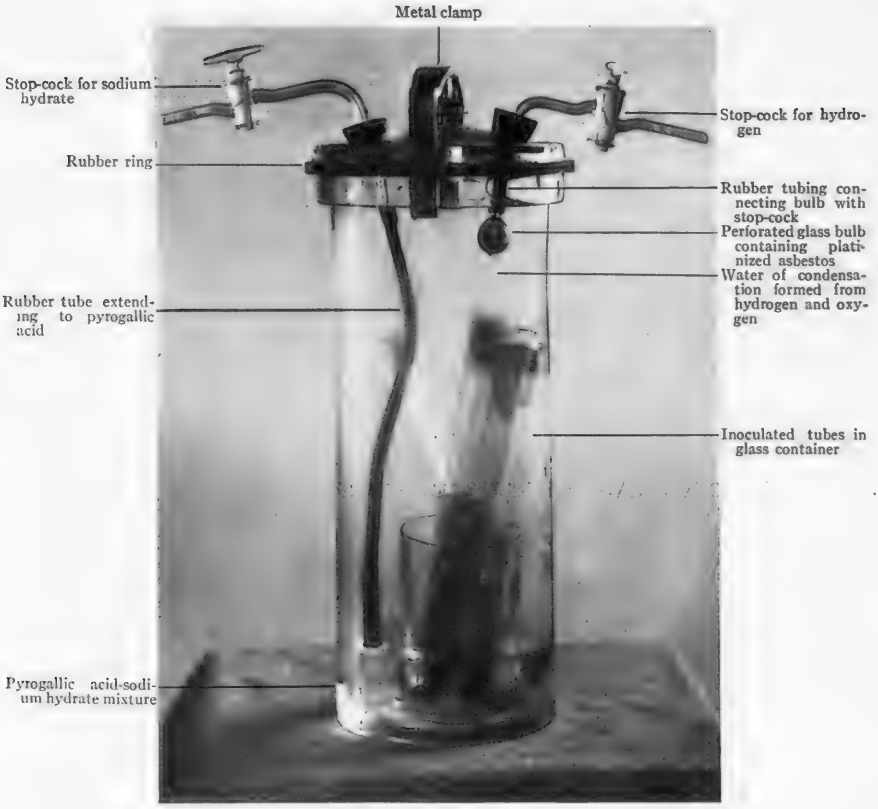

Metal clamp
Stop-cock for sodium hydrate
Rubber ring
Rubber tube extending to pyrogallic acid
Pyrogallic acid-sodium hydrate mixture
Stop-cock for hydrogen
Rubber tubing connecting bulb with stop-cock
Perforated glass bulb containing platinized asbestos
Water of condensation formed from hydrogen and oxygen
Inoculated tubes in glass container

FIG. 1.

METHODS FOR THE DETERMINATION OF PNEUMOCOCCUS TYPES.*

By FRANCIS G. BLAKE, M.D.

(*From the Hospital of The Rockefeller Institute for Medical Research.*)

(Received for publication, March 10, 1917.)

It has been shown by Cole[1] and his associates that 75 to 80 per cent of cases of lobar pneumonia in adults are caused by three fixed types of highly parasitic pneumococci which can be differentiated readily by immunological methods, and that the remaining 20 to 25 per cent of cases are caused by a heterogeneous group of serologically independent varieties of pneumococci. These types have been classified by Dochez and Gillespie[2] primarily by means of animal protection experiments into Groups I, II, III (*Pneumococcus mucosus*), and IV (heterogeneous group). It has been shown that classification by agglutination corresponds to the classification by animal protection. Avery[3] has further shown that there is a considerable group of pneumococci which are related to Group II organisms in that mice are protected against infection with such atypical Group II pneumococci by Type II antipneumococcus immune serum and in that such pneumococci are agglutinable by Type II immune serum, though less rapidly and completely than Type II pneumococci. Immune sera prepared by the immunization of animals against such atypical Group II pneumococci, however, fail to agglutinate Type II organisms or to protect mice against infection with Type II strains. These atypical Group II pneumococci have been classified as Subgroups IIa, IIb, and IIx (heterogeneous). No such atypical strains have been encountered in Groups I or III. Statistical studies have shown that the mortality rate in pneumonia varies with the type of pneumococcus causing the disease, the percentage of deaths in each group being fairly constant from year to year. In a series of 400 cases[4] not receiving specific serum treatment the mortality rate was as follows: Group I, 25.2 per cent; Group II, 28 per cent; Group III, 56 per cent; Group IV, 14 per cent. These facts have be-

* This work was done under the tenure of a William O. Moseley Travelling Fellowship from Harvard University.

[1] Cole, R., *Arch. Int. Med.*, 1914, xiv, 56; *N. Y. Med. J.*, 1915, ci, 1, 58; *Tr. Cong. Am. Phys. and Surg.*, 1916, x, 138.

[2] Dochez, A. R., and Gillespie, L. J., *J. Am. Med. Assn.*, 1913, lxi, 727.

[3] Avery, O. T., *J. Exp. Med.*, 1915, xxii, 804.

[4] Moore, H. F., and Chesney, A. M., *Arch. Int. Med.*, 1917, xix, 611.

come a valuable guide in prognosis and have provided a basis for rational specific serum therapy in lobar pneumonia which at present has been successfully developed for cases of pneumonia caused by Type I pneumococci.

The determination of pneumococcus types in cases of lobar pneumonia is rapidly coming into extended use because of its value as a prerequisite for specific serum treatment and in the field of prognosis. It is essential that a reliable and standard technique for the determination of types should be used and that the method should be as rapid as possible in order that serum treatment, when indicated, may be instituted at the earliest possible moment.

The method in common use consists in the intraperitoneal injection into a mouse of a specimen of the patient's sputum. By this means a rapid growth of the pneumococcus is obtained while other secondary organisms are usually inhibited. After a suitable interval, varying in the individual case from 5 to 24 hours as determined by preliminary peritoneal puncture with a capillary pipette, the mouse is killed and the peritoneal exudate is washed out with 4 to 5 cc. of normal salt solution. The leukocytes and fibrin are removed from the peritoneal washings by centrifugalization at low speed; the supernatant bacterial suspension is decanted into a second centrifuge tube and whirled at high speed until the bacteria are thrown down. This supernatant fluid is discarded, and the bacterial sediment resuspended in normal salt solution. The type of pneumococcus present is then determined by macroscopic agglutination tests with undiluted antipneumococcus immune serum. At the time of mouse autopsy cultures of the peritoneal exudate and heart's blood are made in broth and on blood agar plates for subsequent confirmation of the determination of type made on the peritoneal washings.

Certain factors have interfered with the rapid determination of types by this method in an appreciable number of cases. The most frequent difficulty encountered has been the growth of secondary organisms in the peritoneal exudate together with the pneumococcus, notably *Bacillus influenzæ* and less frequently *Micrococcus catarrhalis*, staphylococci, streptococci, and *Bacillus typhi murium*. When this occurs agglutination of the pneumococci present is either inhibited or markedly delayed, or spontaneous agglutination occasionally occurs in all tubes. A delay of 18 to 24 hours results until ag-

glutination tests can be made on pure cultures of the pneumococcus obtained from cultures made of the heart's blood at the time of mouse autopsy. When *Bacillus influenzæ* or *Bacillus typhi murium* are present in the peritoneal exudate they are usually present also in the heart's blood cultures and further delay takes place through the necessity of fishing and culturing pneumococcus colonies from the blood agar plate.

A further difficulty which has been encountered recently with the agglutination technique commonly used has been the occurrence of a small number of strains of pneumococci properly belonging in Group IV which agglutinate in all three types of antipneumococcus serum. The character of the agglutination is less rapid and less complete than that which occurs with the fixed types of pneumococci, resembling the agglutination of Subgroup II pneumococci by Type II serum. This phenomenon of cross agglutination is one that might be expected from our knowledge of other bacterial groups and it in no way invalidates the classification of the parasitic pneumococci into sharply defined immunological groups. It rather indicates that with the use of undiluted serum of high agglutinin titer a zone of non-specific reaction is encountered. While only a relatively small number of pneumococcus strains exhibit the phenomenon of cross agglutination, it has been shown by the more delicate precipitin test that a limited zone of non-specific reaction exists for strains of pneumococci of all four groups. It is necessary to give but one protocol of a considerable series to demonstrate this (Table I).

TABLE I.

Determination of the Zone of Non-Specific Precipitin Reaction, Using Pneumococcus Type II Antigen and Antipneumococcus Immune Sera I, II, and III.

Type II antigen 0.5 cc.	1:100	1:500	1:1,000	1:5,000	1:10,000	1:50,000	1:100,000	1:300,000	1:500,000
Serum I (1:10) 0.5 cc.....	++	±+	+	±	−	−	−	−	−
" II (1:10) 0.5 "	+++	+++	+++	+++	+++	++	+±	±	−
" III (1:10) 0.5 "	++	++	+±	+	±	−	−	−	−
Normal horse serum (1:10) 0.5 cc....	−								

The reactions were read after 2 hours at 37°C. and over night on ice. Pneumococcus II antigen was prepared by drying *in vacuo* the washed pneumococci from an 18 hour 1,000 cc. bouillon culture. The dried bacterial bodies were taken up in salt solution (10 mg. per cubic centimeter) and the suspension was repeatedly frozen and thawed until a faintly opalescent fluid free from bacterial bodies was obtained. Dilutions of the antigen were made in 0.85 per cent salt solution.

From Table I it is evident that a zone of non-specific precipitation occurs with Pneumococcus II antigen in dilutions not greater than 1:10,000 and that when higher dilutions are used the reaction becomes specific. Similar results have been obtained with antigen prepared from strains of Pneumococcus Type I and Pneumococcus Type III. Group IV pneumococcus antigens show a precipitin reaction only within the limits of the non-specific zone.

To obviate these difficulties certain improvements in the technique for the determination of pneumococcus types have been developed and will be presented below. The preliminary steps are the same as in the commonly used method and will be given in detail for the sake of completeness.

Inoculation of Mice with Sputum.

Collection of Sputum.—Care should be exercised in the collection of sputum to obtain a specimen from the deeper air passages as free as possible from saliva. This can be done in practically all cases, even the most difficult, with a little persistence. The physician or nurse should personally superintend the collection of sputum inducing the patient to cough until a suitable specimen is raised, care being taken not to allow the patient to swallow the lung sputum. The sputum is collected in a sterile Esmarch dish or other suitable container and should be sent at once to the laboratory for mouse injection. When delay is unavoidable the specimen should be kept on ice during the interval.

Microscopic Examination of the Sputum.—Direct films are made from the sputum and stained by Gram's method, with 10 per cent aqueous saffranin as a counterstain, by Ziehl-Neelson's stain, and

by Hiss's capsule stain. This serves to give an idea of the nature of the organisms present and an indication of the source of the sputum. Suitable lung specimens are relatively free, in most instances, from contaminating mouth organisms. It is frequently possible to identify Type III (*Pneumococcus mucosus*) organisms when they are present, as they possess a very large distinct capsule staining by both Gram's and Hiss's methods.

Mouse Inoculation.—A small portion of the sputum, about the size of a bean, is selected and washed through three or four changes of sterile salt solution in sterile Esmarch or Petri dishes to remove surface contaminations. The washed sputum is then transferred to a sterile mortar, ground up, and emulsified with about 1 cc. of sterile bouillon or salt solution, added drop by drop, until a homogeneous emulsion is obtained that will readily pass through the needle of a small syringe. 0.5 to 1 cc. of this emulsion is inoculated intraperitoneally into a white mouse with a sterile syringe. The pneumococcus grows rapidly in the mouse peritoneum while the majority of saprophytic mouth organisms rapidly die off with the exceptions noted above, *Bacillus influenzæ*, and occasionally *Micrococcus catarrhalis*, staphylococci, and streptococci. Pneumococcal invasion of the blood stream also occurs early. *Bacillus influenzæ* like wise invades the blood stream if present; other organisms as a rule do not.

The time elapsing before there is a sufficient growth of the pneumococcus in the mouse peritoneum for the satisfactory determination of type varies with the individual case, depending upon the abundance of pneumococci in the specimen of sputum and the virulence and invasive power of the strain present. It may be from 5 to 24 hours, averaging 6 to 8 hours with the parasitic fixed Types I, II, and III. As soon as the injected mouse appears sick a drop of peritoneal exudate is removed by means of peritoneal puncture with a sterile capillary pipette, spread on a slide, stained by Gram's method, and examined microscopically to determine whether there is an abundant growth of the pneumococcus present. If there is an abundant growth of the pneumococcus present in pure culture the mouse is killed and the determination of type proceeded with. If the growth is only moderate or if other organisms are present in any quantity, further time must be allowed until subsequent examination of the

peritoneal exudate shows an abundant growth of the pneumococcus. It should be emphasized that undue haste in killing the mouse is time lost in the end.

Mouse Autopsy.—As soon as the mouse is killed or dies, the peritoneal cavity is opened with sterile precautions and cultures are made of the exudate in plain broth and on one-half of a blood agar plate. Films are made and stained for microscopic examination by Gram's stain and Hiss's capsule stain. The peritoneal exudate is then washed out by means of a sterile glass pipette with 4 to 5 cc. of sterile salt solution, the washings being placed in a centrifuge tube. Cultures are then made from the heart's blood in plain broth and on the other half of the blood agar plate.

Determination of Type.

Agglutination Method.—When the pneumococcus is present in pure culture in the peritoneal exudate the determination of type may be satisfactorily made by macroscopic agglutination tests as follows. The peritoneal washings are centrifugalized at low speed for a few minutes until the cells and fibrin contained in the exudate are thrown down. The supernatant bacterial suspension is decanted into a second centrifuge tube and centrifugalized at high speed until the organisms are thrown down. The supernatant fluid is discarded and the bacterial sediment taken up in sufficient sterile salt solution to make a moderately heavy suspension. The concentration of bacteria should be similar to that of a good 18 hour broth culture of the pneumococcus. This suspension is used directly for macroscopic agglutination tests, being mixed with immune serum in small test-tubes in equal quantities of 0.5 cc. each.

To obviate the difficulty that occasionally arises from the occurrence of Group IV strains that show cross agglutination in all three types of immune serum, the optimum dilutions of serum and the optimum incubation time that will surely identify all type strains and fail to give any cross agglutination reactions have been determined on a large series of strains.[5] The results are shown in Table II.

[5] This work applies only to the antipneumococcus immune serum prepared at the Hospital of The Rockefeller Institute for Medical Research.

TABLE II.

Determination of Pneumococcus Types by Agglutination.

Pneumococcus suspension 0.5 cc.	Serum I (1:20) 0.5 cc.	Serum II (undiluted) 0.5 cc.	Serum II (1:20) 0.5 cc.	Serum III (1:5) 0.5 cc.
Type I	++	−	−	−
" II	−	++	++	−
Subgroups II a, b, x	−	+	−	−
Type III	−	−	−	++
Group IV	−	−	−	−

Incubation for 1 hour at 37°C.

From Table II it will be seen that a 1:20 dilution of Type I serum, making with the addition of an equal amount of pneumococcus suspension a final dilution of 1:40, a 1:20 dilution of Type II serum making a final dilution of 1:40, and a 1:5 dilution of Type III serum making a final dilution of 1:10, serve to agglutinate Types I, II, and III pneumococci respectively and fail to show any cross agglutination reactions with strains belonging in Group IV. It will further be seen that with 0.5 cc. of undiluted Type II serum, as well as with the 1:20 dilution, pneumococci belonging to the various Subgroups II may be identified and rapidly differentiated from Type II pneumococci in that they show partial to complete agglutination in undiluted Type II serum, but not in the 1:20 dilution at the end of 1 hour's incubation at 37°C.

For the determination of types of pneumococci in the peritoneal washings such serum dilutions give the most satisfactory and clear-cut results. Five small test-tubes are set up as follows: Tube 1, 0.5 cc. of Serum I (1:20) + 0.5 cc. of bacterial suspension; Tube 2, 0.5 cc. of Serum II (undiluted) + 0.5 cc. of bacterial suspension; Tube 3, 0.5 cc. of Serum II (1:20) + 0.5 cc. of bacterial suspension; Tube 4, 0.5 cc. of Serum III (1:5) + 0.5 cc. of bacterial suspension; Tube 5, 0.1 cc. of sterile ox bile + 0.3 cc. of bacterial suspension to determine the bile-solubility of the strain for differentiation from the streptococcus. The tubes are incubated in the water bath for 1 hour at 37°C. Agglutination of Types I, II, and III pneumococci in such serum dilutions is practically always immediate in the homologous serum and no agglutination occurs in the heterologous sera.

Rapid clumping of the organisms is seen to take place and may be brought out clearly by gentle agitation of the tubes. For the identification of Subgroup II pneumococci incubation is necessary, such strains showing partial to complete agglutination in undiluted Type II serum at the end of 1 hour's incubation. If no agglutination occurs and the organism is bile-soluble, it is classified as a Group IV pneumococcus.

Precipitin Method.—It has been stated above that the determination of pneumococcus types by macroscopic agglutination tests with the peritoneal washings is interfered with when other organisms are present, with a resultant delay of 18 hours or more before the type of pneumococcus present can be established. To obviate this difficulty the following method has been devised. Dochez and Avery[6] have shown that the pneumococcus produces in broth cultures during the period of active growth a soluble substance which gives a specific precipitin reaction with the homologous antipneumococcus immune serum. It seemed probable that this soluble substance or precipitinogen would be present in the peritoneal exudate of the mouse in sufficient quantity to give a specific precipitin reaction with the homologous serum and such has proved to be the case. The method to be described is dependent upon this phenomenon.

The peritoneal exudate is washed out with 4 to 5 cc. of sterile salt solution by means of a sterile glass pipette and placed in a centrifuge tube. The peritoneal washings containing cells, fibrin, and bacteria are immediately centrifuged at high speed until the supernatant fluid is water clear. The supernatant fluid is then pipetted off, with care not to disturb the sediment, which is discarded, and is mixed in quantities of 0.5 cc. each with an equal amount of the antipneumococcus immune serum in a series of small test-tubes as follows: Tube 1, 0.5 cc. of Serum I (1:10) + 0.5 cc. of supernatant peritoneal washings; Tube 2, 0.5 cc. of Serum II (undiluted) + 0.5 cc. of supernatant peritoneal washings; Tube 3, 0.5 cc. of Serum II (1:10) + 0.5 cc. of supernatant peritoneal washings; Tube 4, 0.5 cc. of Serum III (1:5) + 0.5 cc. of supernatant peritoneal washings. An immediate specific precipitin reaction occurs in the tube contain-

[6] Dochez, A. R., and Avery, O. T., *Proc. Soc. Exp. Biol. and Med.*, 1916–17, xiv, 75.

ing the homologous immune serum, the other tubes remaining clear (Table III). No incubation is necessary. Two tubes of Type II serum are used for the purpose of distinguishing between Type II pneumococci and members of the Subgroups II, the former giving a precipitin reaction in both tubes, the latter only in the undiluted Type II serum. A negative reaction in all tubes indicates a pneumococcus belonging in Group IV.

The method has been tested with a large number of strains and has been consistently positive and specific with pneumococci of Types I, II, and III and consistently negative with pneumococci of Group IV. The presence of other organisms together with the pneumococcus in the peritoneal exudate does not interfere with the reaction and other organisms than the pneumococcus produce no substance that might give a false positive reaction.

TABLE III.

Determination of Pneumococcus Types by the Precipitin Method.

Supernatant peritoneal washings 0.5 cc.	Serum I (1:10) 0.5 cc.	Serum II (undiluted) 0.5 cc.	Serum II (1:10) 0.5 cc.	Serum III (1:5) 0.5 cc.
Type I	++	−	−	−
" II	−	++	++	−
Subgroups II a, b, x	−	+	−	−
Type III	−	−	−	++
Group IV.	−	−	−	−

The results with Subgroup II pneumococci have not been so satisfactory. Reference to Table III will show that pneumococci belonging to these groups give a precipitin reaction with undiluted Type II serum but not with the 1:10 dilution, thereby being distinguished from Type II pneumococci. A number of Subgroup II organisms, however, have been encountered in which the peritoneal washings have failed to give a precipitin reaction with undiluted Type II serum. In the identification of the fixed parasitic types of pneumococci this occasional difficulty is of little practical importance from the point of view of treatment as there is at present no specific therapy for cases of pneumonia caused by pneumococci of the Subgroups II. For purposes of classification and statistics these organisms can be

readily identified subsequently when the organism has been obtained in pure culture.

In order to determine the shortest time after mouse inoculation in which a clear-cut positive precipitin reaction may be obtained and whether it occurs as soon as a satisfactory agglutination test can be made, a series of mice have been inoculated intraperitoneally with measured amounts of pure pneumococcus cultures and the mice have been killed at varying intervals. Simultaneous determinations of type have been made by both the agglutination and precipitin methods on each peritoneal washing. The results are shown in Table IV.

TABLE IV.

Comparison of Agglutination and Precipitin Methods.

Mouse.	Time killed after inoculation with 0.01 cc. of culture.	Series A, Type I.		Series B, Type II.		Series C, Type III.	
		Agglutination.	Precipitin.	Agglutination.	Precipitin.	Agglutination.	Precipitin.
	hrs.						
1	4	−	−	−	−	−	−
2	5	−	−	−	−	−	+
3	6	−	−	−	+	−	++
4	7	−	+	−	++	−	++
5	8	++	++	++	++	++	++

From these experiments it is evident that the agglutination method possesses no advantage in point of time over the precipitin method. The presence of the soluble precipitinogen in the peritoneal exudate in sufficient quantity to give a clear-cut precipitin reaction coincides in the case of Type I pneumococci with the earliest time when satisfactory agglutination tests can be made. With Type II pneumococci it occurs earlier and with Type III still earlier. This phenomenon exhibits an interesting parallelism with the capsular formation and virulence of the three types of pneumococci.

The precipitin method possesses the following distinct advantages. It is available as soon as satisfactory agglutination tests can be made; incubation of the tubes is unnecessary; it is not interfered with by the presence of other organisms in the exudate; it is specific and shows no cross immunity reactions; it is applicable to mice which

through unavoidable circumstances have been dead for some time before the determination of type can be made and in which autolysis of the pneumococci or postmortem invasion of the peritoneal cavity by other organisms has made the agglutination method impracticable. For these reasons it is recommended as the method of choice in all cases.

Identification of Type III Pneumococci by Morphological and Cultural Characteristics.—If Type III antipneumococcus immune serum is not available for diagnostic purposes Type III pneumococci may be identified in most instances by cultural and morphological characteristics. *Pneumococcus mucosus* is usually somewhat larger, rounder, and less lanceolate than other types of pneumococci. It possesses a large distinct capsule which stains readily with Hiss's capsule stain and usually retains the pink counterstain with Gram's method. The peritoneal exudate produced on mouse inoculation is usually quite mucoid and colonies on solid media are moist, mucoid, and spreading. It is always bile-soluble. These characteristics usually serve to differentiate Type III pneumococci from other types. Occasional strains of pneumococci which agglutinate in Type III serum, however, are encountered which do not show well developed mucoid characteristics and cannot be distinguished culturally from other types. Furthermore, Type II strains are occasionally met with that exhibit fairly well developed mucoid characteristics. For these reasons the identification of Type III pneumococci by morphological and cultural characteristics is not always absolute, and the diagnosis should be established by immunological methods when Type III serum is available.[7]

Confirmation of Type.

The determination of type on the peritoneal washings should be confirmed by macroscopic agglutination tests with a pure bouillon culture of the pneumococcus obtained from culture of the heart's blood at the time of mouse autopsy. The technique is the same as that employed in the agglutination tests on the bacterial suspension obtained from the peritoneal washings and should include a test for bile-solubility.

[7] Wadsworth, A. B., and Kirkbride, M. B., *J. Exp. Med.*, 1917, xxv, 629.

Determination of Types of Pneumococci in Blood Cultures, Spinal Fluids, Empyema Fluids, and by Lung Puncture.

Blood Culture.—The usual technique in routine blood cultures is carried out. From a positive bouillon blood culture 10 cc. are removed by pipette and centrifugalized at low speed to remove the blood cells. The supernatant fluid is pipetted off and the bacteria are thrown down by centrifugalization at high speed, the supernatant fluid is discarded, and the bacterial sediment is suspended in sterile salt solution. The pneumococcus type is then determined by macroscopic agglutination tests following the same technique described above.

Spinal Fluid and Empyema Fluid.—Cultures are made by the methods ordinarily employed in culturing fluids and the type of pneumococcus is determined when the culture has grown out by the use of the same technique as that applied to blood cultures. If desired, in addition to culturing spinal fluids, a portion of the fluid may be centrifugalized at high speed to throw down the pneumococci present, and the sediment, taken up in 1 cc. of sterile salt solution, inoculated intraperitoneally into a mouse.

Lung Puncture.—This procedure should be resorted to only when it is impossible to obtain a suitable specimen of sputum or a positive blood culture. Cultures are made in bouillon of the lung puncture material and the determination of type is made by the same technique as that employed in the case of blood cultures.

Determination of Pneumococcus Types by Direct Sputum Culture.

It was thought that the determination of types of pneumococci by the precipitin method might be possible by direct culture of the washed sputum in bouillon without resort to mouse passage. Tubes of bouillon were inoculated with specimens of sputum and incubated 6 to 8 hours at 37° C. The cultures were then centrifugalized at high speed until the supernatant bouillon was clear, and precipitin tests were made by mixing equal parts of the supernatant bouillon and immune serum. In a few instances sufficient precipitinogen had been produced by the growth of the pneumococcus in the culture to give a positive precipitin reaction with the homologous serum. This by

no means invariably occurred with all specimens of sputum, however, and seemed to depend largely upon the number of pneumococci present in the sputum and to some extent upon the type of pneumococcus. The number of experiments done was small and the results were not sufficiently satisfactory to make the method of practical use. It is possible that further experiments along these lines might develop a technique which would prove available for use as a routine method.

SUMMARY.

The determination of pneumococcus types in lobar pneumonia is of value in the field of prognosis and as a prerequisite for specific serum therapy. The method for the determination of types should be as rapid as possible and a standard technique should be employed. The most satisfactory method is by the intraperitoneal inoculation of a mouse with the patient's sputum, by which means a rapid and abundant growth of the pneumococcus is obtained and secondary organisms are rapidly eliminated in most instances. The diagnosis of type is made directly on the peritoneal exudate. Certain factors in the method commonly used have interfered with the rapid determination of types in an appreciable number of cases, notably the growth of other organisms in the peritoneal exudate together with the pneumococcus, and some confusion has arisen because occasional strains of pneumococci have been encountered that show cross agglutination reactions when undiluted immune serum is used. Such reactions have been shown to be due to a limited zone of non-specific immunity and they in no way invalidate the classification of the pneumococci into sharply defined immunological groups. The optimum dilutions of serum have been determined that will agglutinate all type strains of pneumococci and fail to cause any cross agglutination reactions when mixed with equal amounts of pneumococcus cultures and incubated for 1 hour at 37°C. They are a 1:20 dilution of Serum I, a 1:20 dilution of Serum II, and a 1:5 dilution of Serum III. For the diagnosis of Subgroup II pneumococci undiluted Type II serum is required.

To obviate the other difficulties of the method commonly used a new method for the determination of types has been devised. It

depends upon the fact that there is produced by the growth of the pneumococcus a soluble substance which is present in the peritoneal exudate of the mouse in sufficient quantity to give a specific precipitin reaction with the homologous immune serum. The precipitin method can be used in all instances in which the determination of types by the agglutination method is possible, and it possesses certain distinct advantages which make it available when the agglutination method is impracticable. It is of particular value as a time-saving device in those instances where the presence of other organisms together with the pneumococcus in the peritoneal exudate causes a delay of 18 hours or more before the type of pneumococcus can be definitely established. It is therefore recommended as the method of choice in all cases. If desired, both the agglutination and precipitin methods may be applied to the same specimen of peritoneal washings.

CICATRIZATION OF WOUNDS.

V. New Mathematical Expression of Cicatrization.

By A. JAUBERT de BEAUJEU, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York, and Hospital 21, Compiègne, France.)

(Received for publication, November 24, 1916.)

A new formula with two equations gives in mathematical terms Carrel and Hartmann's law:[1] "the rate of cicatrization diminishes at the same time as the size but less rapidly."

In the time dt, the area cicatrized, dS, is proportional to S:

$$dS = -\lambda S dt \quad \text{or} \quad \frac{dS}{dt} = -\lambda S$$

λ is positive and the formula shows that the rate, $\frac{dS}{dt}$, decreases with S.

By integration we get

$$\int \frac{dS}{S} = -\lambda \int dt$$

$$(1) \qquad \text{Log}_e S = -\lambda t + \text{Log}_e S_o \qquad \text{or} \qquad S = S_o e^{-\lambda t}$$

where S_o is the initial area.

If the coefficient λ is constant, the law of cicatrization can be expressed by simple logarithmic formula.

The rate of cicatrization decreases less rapidly than the size; that is, λ is not constant and must increase slightly when the area decreases. In the time dt the variation of λ, $d\lambda$, is proportional to λ:

$$d\lambda = \mu \lambda dt \quad \text{or} \quad \frac{d\lambda}{dt} = \mu \lambda$$

If μ is positive, the equation indicates that λ increases because the derivative $\frac{d\lambda}{dt}$ is positive.

[1] Carrel, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

By integration we get

$$\int \frac{d\lambda}{\lambda} = \mu \int dt$$

$$(2) \qquad \mathrm{Log}_e \lambda = \mu t + \mathrm{Log}_e \lambda_o \qquad \text{or} \qquad \lambda = \lambda_o e^{\mu t}$$

where λ_o is the initial value of λ. λ is calculated by equation (2) and with this value of λ we can obtain S by the equation (1).

The two coefficients λ_o and μ may be determined to make the values calculated and observed correspond.

The area at any time can be obtained immediately without calculating the intermediate areas.

CICATRIZATION OF WOUNDS.

VI. Bacteriological Asepsis of a Wound.

By A. VINCENT, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research, New York, and Hospital 21, Compiègne, France.*)

(Received for publication, February 9, 1917.)

In a previous communication[1] it has been shown that even a slight infection prevents normal cicatrization. Experiments on cicatrization must be carried out on surgically aseptic wounds. The degree of asepsis can easily be obtained by the method already described.[2] Surgical asepsis differs widely from bacteriological asepsis. Disinfected wounds which unite by first intention still contain numerous bacteria, particularly *Micrococcus epidermidis albus*. However, the experiments carried out by Carrel and Hartmann have shown that the new method of wound sterilization frequently leads to bacteriological asepsis.

The object of the following experiments was to determine to what degree wounds irrigated with Dakin's solution or treated with chloramine paste become bacteriologically aseptic.

Preventive Action of Dakin's Hypochlorite Solution and of Chloramine-T.

In the first series of experiments we undertook to ascertain what quantity of Dakin's solution or of chloramine-T is necessary to retard or prevent the growth of staphylococcus. This preliminary experiment was necessary in order to determine whether the quantity of antiseptic taken from the surface of the wound at the same time as the secretions was strong enough to inhibit the growth of the bacteria contained in the secretions.

[1] Carrel, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

[2] Carrel, A., and Dehelly, G., Le traitement des plaies infectées, Paris, 1916.

To 5 cc. of broth are added 0.1 to 2 cc. of Dakin's solution or of chloramine-T solution. A drop of a 24 hour broth culture of staphylococcus is then added. The staphylococcus used was obtained from a case of suppurative arthritis of the wrist treated during the preceding 15 days with Dakin's solution. Tables I to IV give the results of the experiments. They show that the power of Dakin's solution to restrain growth becomes evident after 12 hours if more than 0.4 cc. is used. In the same way, 0.3 cc. of 1 per cent chloramine-T also retards the growth. This power is relatively weak. The amount of Dakin's solution or of chloramine-T necessary to prevent completely the growth of the staphylococcus in 5 cc. of broth is, therefore, relatively large. 2 cc. of Dakin's solution or more than 1 cc. of 1 per cent chloramine-T are necessary for sterilization of the media.

TABLE I.

Antiseptic Properties of Dakin's Solution. First Test.

In 5 cc. of broth.	Cc. of Dakin's solution.															Control.
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	2.0	
days																	
1	+	+	+	+	+	+	Weak.	?	−	−	−	−	−	−	−	−	+
2	+	+	+	+	+	+	+	+	+	+	−	+	−	−	−	−	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+

TABLE II.

Second Test.

In 5 cc. of broth.	Cc. of Dakin's solution.								Control.
	0.9	1.0	1.1	1.2	1.3	1.4	1.5	2.0	
hrs.									
6		−	−	−	−	−	−	−	Weak.
12		−	−	Weak.	−	−	−	−	+
24	+	−	−	+	Weak.	−	−	−	+
48	+	−	+	+	+	+	+	−	+

TABLE III.

Restraining Power.

In 5 cc. of broth.	Cc. of Dakin's solution.										Control.
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	
hrs.											
6	+	Weak.	Very weak.	?	−	−	−	−	−	−	+
12	+	+	Weak.	+	Weak.	−	−	−	−	−	+
24	+	+	+	+	+	+	+	−	+	−	+
36	+	+	+	+	+	+	+	+	+	+	+

TABLE IV.

Antiseptic Properties of 1 Per Cent Chloramine-T.

In 5 cc. of broth.	Cc. of chloramine-T.															Control
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	2.0	
hrs.																	
6	Weak.	Weak.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Weak.
12	+	+	?	−	−	?	−	−	−	−	−	−	−	−	−	−	+
18	+	+	Weak.	−	?	Weak.	−	−	−	?	−	−	−	−	−	−	+
24	+	+	+	?	+	+	?	+	−	Weak.	−	−	−	−	−	−	+
36	+	+	+	+	+	+	+	+	−	+	−	−	−	−	−	−	+
48	+	+	+	+	+	+	+	+	−	+	−	−	−	−	−	−	+
3 days.	+	+	+	+	+	+	+	+	−	+	−	−	−	−	−	−	+

These experiments show that the small quantity of antiseptic removed with the bacteria by the platinum loop does not affect the growth of the cultures described below.

Bacteriological Examination of Surgically Aseptic Wounds.

As soon as films taken on several consecutive days showed that no bacteria were present, cultures were made from different parts of the wound. Before taking the cultures the irrigation was suspended for 2 hours, and in cases in which the chloramine paste had been used, all trace of the paste was carefully removed with distilled water and the wound was swabbed with sterilized gauze. It is advisable when taking the films to scrape the surface of the wound lightly with a platinum loop, care being taken to avoid making the granulations bleed.

TABLE V.

Results of Experiments.

Case No.	Wound.	Treatment.	Duration of treatment.	Result of culture.	Bacteria recovered.	Result.
			days			
1	Complicated fracture of right humerus.	Dakin's solution.	7	+	*B. pyocyaneus* and various cocci.	Closure and suture 6 days after culture. Healing *per primam.*
2	Flesh wound of right knee.	"	7	+	*Staphylococcus albus* and an unknown bacillus.	"
3	Flesh wound of left leg.	"	7	+	*Micrococcus epidermidis albus* and *B. pyocyaneus.*	"
4	Flesh wound of right thigh.	"	8	+	Enterococcus, *Staphylococcus albus*, and various diplococci.	Closure and suture 4 days after culture. Healing *per primam.*
5	Perforated wound with fracture of right humerus.	"				
	(*a*) External wound.		8	−		Closure and suture on day of subculture. Healing *per primam.*
	Anaerobic culture.		11	−		
	Subculture.		11	−		
	(*b*) Internal wound.		8	+	*Micrococcus epidermidis albus.*	
6	Complicated fracture of neck of left femur.	"	12	+	*Staphylococcus aureus*, *Micrococcus epidermidis albus*, and various diplococci.	Closure and suture 13 days after culture. Healing *per primam.*
7	Complicated fracture of right tibia.	"	42	+	*Staphylococcus aureus* and *albus.*	Closure and suture on day of culture. Sloughing on 2 cm. of the suture.
8	Surface wound following an old fracture of tibia.	Dakin's solution and chloramine-T.	109	−		Surface granulation.
	Anaerobic culture.		112	−		
	Subculture.		112	−		
	Second culture.		117	+	*Micrococcus albus* and bacillus like *Proteus vulgaris.*	

TABLE V—*Continued.*

Case No.	Wound.	Treatment.	Duration of treatment.	Result of culture.	Bacteria recovered.	Result.
			days			
9	Wound of dorsal surface of foot following an old fracture of fifth metatarsal. (Wound heavily infected; gaseous gangrene.)	Dakin's solution and chloramine-T.	109	+	*Staphylococcus aureus*, *B. pyocyaneus*, and an unknown bacillus.	Surface granulation.
10	Flesh wound of calf.	"	117	+	*Staphylococcus aureus* and *albus* and an unknown bacillus.	"
11	Wound of dorsal surface of foot above fracture of metatarsus.	"	37	−		"
	Anaerobic culture.		40	−		
	Subculture.		40	−		
12	Complicated fracture of fourth metatarsal.	"	23	−		"
	Anaerobic culture.		26	−		
	Subculture.		26	−		
	Second culture.		32	−		
13	Open wound of leg after ligature of popliteal and suture of posterior tibial arteries.	Dakin's solution.	20	+	*Micrococcus epidermidis albus.*	Closure and suture on day of culture. Healing *per primam.*
14	Flesh wound of right arm.	"	8	−		Surface granulation.
	Anaerobic culture.		11	−		
	Subculture.		11	−		
15	Large wound with fracture of shoulder blade.	"				After slight reaction, inflammation on upper border of wound, redness for 3 days, and small hematoma surrounding the suture.
	(*a*) Upper part of wound.		16	+	*Staphylococcus albus.*	
	(*b*) Lower part of wound.		16	−		
	Subculture.		19	−		

TABLE V—*Concluded.*

Case No.	Wound.	Treatment.	Duration of treatment.	Result of culture.	Bacteria recovered.	Result.
			days			
16	Flesh wound of calf with lymphangitis.	Dakin's solution.	19	+	*Micrococcus albus* and an unknown diplococcus.	Closure and suture on day of culture. Healing *per primam.*
17	Wound of left arm with comminuted fracture of humerus.	Dakin's solution and chloramine. T.	19	+	Streptococcus and *Staphylococcus albus.*	Surface granulation.
18	Flesh wound of right leg.	Chloramine-T.	20	−		"
	Anaerobic culture.		23	−		
	Subculture.		23	−		
19	Wound of left hand with complete destruction of fourth and fifth metacarpals.	"	23	+	*Staphylococcus aureus* and *albus.*	"
20	Wound of left knee following arthrotomy.	Dakin's solution and chloramine-T.	98	−		"
	Anaerobic culture.		101	−		
	Subculture.		101	−		
	Second culture.		110	−		

The tubes of broth were inoculated and incubated for 15 days at 36°C. If the culture remained sterile, a subculture was made on the 3rd day. At the same time, a fresh film was taken at the surface of the wound for an anaerobic culture in glucose broth with an upper layer of paraffin oil. Broth has proved an excellent medium for the bacteria found in wounds. Twenty cases were subjected to the usual treatment with Dakin's solution or chloramine paste (Table V).

SUMMARY.

Of twenty infected cases treated with Dakin's solution or chloramine paste, seven were bacteriologically sterile. This proves that, in general, 35 per cent of the cases thus treated become bacteriologically aseptic. This degree of asepsis is not necessary in order to suture the wound, the absence of bacteria in films being sufficient. Complete sterilization of wounds, can, therefore, now be accomplished.

CICATRIZATION OF WOUNDS.

VII. The Use of Chloramine-T Paste for the Sterilization of Wounds.

By MAURICE DAUFRESNE, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York, and Hospital 21, Compiègne, France.)

(Received for publication, February 9, 1917.)

It has been shown in a previous communication[1] that a wound cicatrizes rapidly if the surface is sterile. If it is more or less infected, the rate of cicatrization is slow or the wound enlarges. In order to obtain a convenient method for the sterilization of wounds we have endeavored to prepare an antiseptic paste which will retain its aseptic properties.

It has been found[1] that ointments and other fatty substances are inefficient when applied to wounds, because the bacteria and antiseptic are covered with fatty material which isolates them from each other and permits the bacteria to multiply freely. Hence the antiseptic paste must be soluble, and the bactericidal agent must be embodied in a substrate suitably chosen so that the whole constitutes a system physically homogeneous. On the other hand, to enable the antiseptic to act continuously the base should be absorbed slowly by the tissues in order to renew the surface of contact constantly, Neutral sodium stearate was used for this purpose because of the facility with which it is made antiseptic and also because it is not injurious to the tissues. It is well known that the slightly soluble sodium soaps, far from being irritating agents, are, on the contrary, soothing. Moreover, they give pastes sufficiently plastic for the dressing of wounds. One of Dakin's chloramines was selected as the bactericidal agent. After many trials we have used the following formula.

[1] Carrell, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

Neutral sodium stearate	86 gm.
Chloramine-T	4–10 "
Distilled water	1,000 cc.

Of the less soluble sodium soaps it is essential to choose those derived from saturated fatty acids and not having double ethylene linkages. The presence of such groups which readily take up the elements of hypochlorous acid (HClO) causes a rapid disappearance of chloramine. On the other hand, stearic acid is a product of sufficient purity and is easily procured; its sodium salt obtained by boiling the calculated amount diluted with caustic soda is aseptic.

We have tried some pastes less concentrated in stearate, but they separate into two portions; the lower part is watery, and the upper portion richer in stearate so that it has the concentration indicated above, which is a minimum.

We have chosen as an antiseptic to combine with the sodium stearate one of the substances studied by Dakin, known as chloramine-T, which is the sodium salt of toluene sodium *p*-sulfochloramide. The reasons for choosing this substance were its high bactericidal power, the absence of caustic action on the skin, the possibility of an exact estimation of its strength, and its stability at a high temperature, which allows the substances to dissolve in a boiling solution of stearate. The question of using sodium hypochlorite was not considered because this product changes rapidly under the influence of heat, and especially because of the sensitiveness of soap solutions to the action of electrolytes.

Several trials were made with various proportions of chloramine-T. 20 gm. per kilo seem to make the paste irritating, thus rendering it useless. 15 gm. per kilo are tolerated by the wounds but are slightly irritating to the skin and congest the granulations, giving them a purple color. 10 gm. per kilo cause neither irritation nor pain; the wounds tolerate application for weeks. At this concentration the bactericidal action is strong enough to disinfect surface wounds completely, as rapidly as Dakin's solution (sodium hypochlorite 0.50 per cent). At the level of contact of the paste and tissues a thick greenish liquid gradually forms, which is apparently the result of the action of the secretion of the wound upon the paste. Bacteriological examination shows the secretion to be sterile, but it is

important to wash it away every day with neutral sodium oleate before making a fresh application. For wounds in the process of cicatrization, paste with 10 gm. of chloramine-T per kilo seems to retard the repair slightly. If the percentage of chloramine-T is further decreased, the antiseptic action decreases proportionately. With 4 gm. per kilo the action on infected wounds is extremely weak, but those that have been disinfected remain sterile and their cicatrization is normal.

The preparation of chloramine paste is as follows: Boil a liter of distilled water and add 80 gm. of stearic acid. When this has melted, gradually add enough caustic soda to saponify the fatty acid and after complete solution add 4 to 10 gm. of chloramine-T, according to the concentration desired. The mixture is then placed in a mixing machine and shaken until thoroughly cooled. The paste is a smooth snow-white cream. Microscopic examination shows that it is composed of a compact felting of fine needles retaining in capillary suspension a colorless liquid. The sodium stearate is slightly soluble at a cold temperature and produces this crystalline felting which retains in its interstices the antiseptic solution. The paste can be kept either in low glass jars or tin tubes, as tin is not corroded by chloramine-T.

The principal disadvantage of this paste is its poor power of preservation; numerous trials showed that 10 per cent of chloramine-T disappeared per month. Substances which might have rendered the paste more stable were either inefficient or lessened its keeping properties. The stability of the paste is limited by the stability of the solution of chloramine-T because the antiseptic is in solution in the paste.

CONCLUSION.

Dakin's toluene sodium *p*-sulfochloramide, mixed with sodium stearate, forms a paste sufficiently active and stable to be used in the treatment of wounds.

CICATRIZATION OF WOUNDS.

VIII. Sterilization of Wounds with Chloramine-T.

By ALEXIS CARREL, M.D., and ALICE HARTMANN, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York, and Hospital 21, Compiègne, France.)

(Received for publication, February 23, 1917.)

In a previous article[1] it was shown that the presence of bacteria at the surface of a wound retards the normal process of cicatrization. According to the nature and size of the infection, the curve representing cicatrization deviated from the calculated curve.[2] In order to investigate the substances which are capable of influencing tissue repair, it is, therefore, imperative that the wound should be kept in an aseptic condition. No specific influence on the progress of healing could be attributed to the substance experimented with unless the possible action of infection was entirely eliminated.

Sterilization of a wound is easily effected by the application of Dakin's hypochlorite solution at the surface of the tissues under appropriate conditions of concentration and duration.[3] In the experiments to be described, it was attempted to simplify the method by substituting for the instillations of Dakin's hypochlorite solution a paste designed gradually to yield up to the tissues one of Dakin's chloramines contained therein. Investigations were undertaken to ascertain whether this paste would be able to keep a sterile wound in an aseptic condition, as well as to sterilize an infected wound, and whether it would retard tissue repair.

Chloramine Paste.

Some unpublished experiments of Dakin had shown the necessity of avoiding fatty substances in the composition of paste intended for the sterilization of wounds.

[1] Carrel, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

[2] du Noüy, P. L., *J. Exp. Med.*, 1916, xxiv, 451.

[3] Carrel, A., and Dehelly, G., Le traitement des plaies infectées, Paris, 1916.

Dakin mixed with vaseline or lanoline certain antiseptic substances which are insoluble in water. Once these substances were applied to the wounds, their bactericidal power became almost negligible. Carrel also experimented with aqueous solutions of antiseptic substances mixed with lanoline, but they failed to have an influence on the infected wounds. It appeared that the active substance remained in the lanoline and exerted no effect upon the bacteria. Carrel next combined certain antiseptic substances in agar, but found that the consistency of the agar rendered it unsuitable for practical use. The cakes yielded to the tissues the liquid they contained, but they were too brittle and easily destroyed.

It was concluded from these experiments that, in order to be efficient, the bactericidal substance would have to be combined with a fat-free substance capable of being moulded exactly to the surface of the wound. At this point Daufresne[4] succeeded in preparing a paste composed of sodium stearate and of toluene sodium *p*-sulfochloramide, the bactericidal properties of which had been discovered by Dakin and which is named chloramine-T for convenience. This paste is sufficiently firm not to flow away when applied to a wound, and yet fluid enough to be moulded to the anfractuosities of a granulating surface or of a fractured bone. The paste contains 8 per cent of sodium stearate and 4 to 15 parts per 1,000 of chloramine-T.

The paste is placed in sterile glass receptacles from which it is withdrawn at the time of dressing by means of wooden spatulas which have been sterilized in the autoclave. It is also preserved in tin tubes, to the ends of which is attached a rubber tube ending in a tapering glass tube. By means of the tube the paste can be injected into deep wounds or fistulas.

Technique for the Application of the Chloramine Paste.

The chloramine paste is designed to maintain in an aseptic condition wounds which have already been disinfected, or to sterilize slightly infected wounds. It should only be applied to wounds which yield small quantities of secretion, have little or no necrotic tissue, and little or no infection.

Neutral sodium oleate is poured on to the wound and the surrounding skin from a flask with a small opening. The granulations, the epithelial edges, and the skin are gently swabbed with a piece of absorbent cotton attached to a forceps. By this means an excellent

[4] Daufresne, M., *J. Exp. Med.*, 1917, xxvi, 91.

cleansing process is effected. The patient should feel no pain; any suffering indicates either that the sodium oleate is incorrectly prepared or that the cleansing is imperfectly carried out. The sodium oleate is next removed with a plug of cotton soaked in water, and the surface of the skin is dried by carefully applying a compress of absorbent gauze.

A sufficient quantity of chloramine paste is withdrawn from the receptacle by means of a sterilized wooden spatula and applied to the surface of the wound to the thickness of at least 1 cm. It should cover not only the granulations, but also the epithelial edges and part of the surrounding skin. If the wound is deep and anfractuous the tube containing the chloramine paste is introduced into the opening, and sufficient chloramine paste is expressed to fill the cavity. But no pressure should be applied during the process.

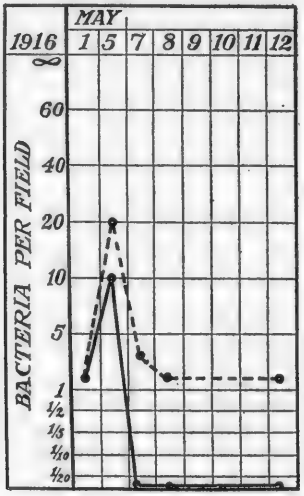
A compress of dry gauze, which should be much larger than the wound itself, is next placed over the chloramine paste. The compress is applied to the surface of the skin and attached to it by means of two or three strips of adhesive plaster. It is important that the gauze should be placed exactly over the wound, for if the bandage is shifted the gauze will introduce bacteria from the surrounding skin on to the surface of the granulations and reinfection will ensue. Above the gauze is placed a piece of absorbent cotton enveloped in gauze. The dressing must not be compressed by bandages and should be renewed every 24 hours. The wound is washed out with sodium oleate every day or two, depending on the condition of the skin. The application of chloramine should be painless; any sensation of pain signifies a technical error on the part of the surgeon.

The bacteriological condition of the wound is examined every day in film preparations of secretions taken from various parts of the wound.

Effect of a Paste Containing 4 Parts per 1,000 of Chloramine-T upon the Bacteriological Condition of an Aseptic or Slightly Infected Wound.

The influence of sodium stearate containing 4 parts per 1,000 of chloramine-T was first tested on surface wounds which had been rendered almost aseptic by instillations of Dakin's hypochlorite solution.

In the first experiment a comparison was made of the effect on the bacteriological condition of a slightly infected wound of sodium stearate alone, and of sodium stearate containing 4 parts per 1,000 of chloramine-T.



TEXT-FIG. 1. Experiment 1. Case 366. Comparative action of sodium stearate and of sodium stearate containing 4 parts per 1,000 of chloramine-T. Wound in back of thigh. The upper part of the wound is dressed with sodium stearate containing 4 parts per 1,000 of chloramine-T. The lower part is dressed with sodium stearate. The upper half of the wound is sterile, while the lower half remains unchanged. The solid line indicates the upper part of the wound; the broken line, the lower part.

Experiment 1. Case 366. Comparison of the Action of Chloramine-T and of Sodium Stearate.—Elongated wound at the back of the thigh.

May 4, 1916. The area of the wound is 23 sq. cm., and the surface averages 1 bacterium per field (Text-fig. 1). The upper half of the wound is dressed with sodium stearate containing 4 parts per 1,000 of chloramine-T, and the lower half is dressed with sodium stearate.

May 5. The upper half of the wound contains 10 bacteria per field, and the lower half 20 per field. Same dressing.

May 6. Same dressing.

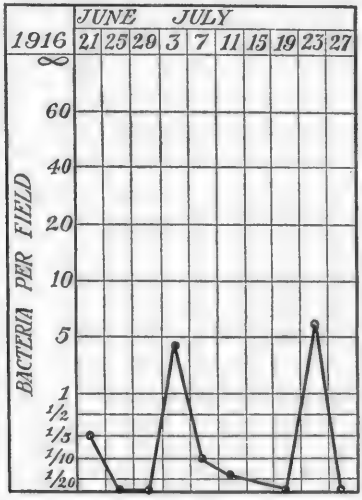
May 7. The upper half of the wound is aseptic; the lower part contains 3 bacteria per field. Same dressing.

May 8. The upper part of the wound is aseptic; the lower half contains 1 bacterium per field. Same dressing.

May 12. The upper half has no bacteria, and the lower half 1 per field.

This observation shows, on the one hand, that sodium stearate has no effect on a slightly infected wound, and, on the other hand, that sodium stearate containing 4 parts per 1,000 of chloramine-T produces surgical asepsis. The bacteria disappeared completely from the films taken from the portions of the wound treated with chloramine-T, whereas they were present in all the films from the part not so treated.

In the following experiments it was attempted to maintain in an aseptic condition wounds which had been rendered surgically sterile at the beginning of treatment.



TEXT-FIG. 2. Experiment 2. Case 488. Preservation of asepsis in a wound by treatment with chloramine-T paste, 4 parts per 1,000. Wound at back of right leg. Two slight reinfections.

Experiment 2. Case 488. Slight Reinfection of a Wound Treated with Chloramine-T, 4 Parts per 1,000.—Wound at back of right leg. Treated with sodium hypochlorite solution.

June 17, 1916. The wound contains but 1 bacterium in 5 fields. Dressing with chloramine-T, 4 parts per 1,000.

June 21. 1 bacterium in 5 fields (Text-fig. 2).

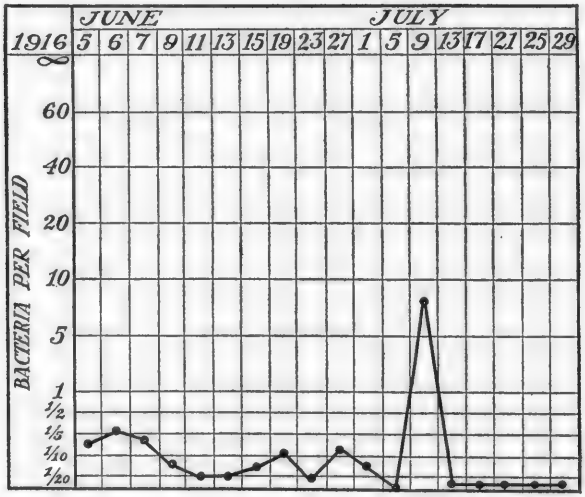
June 25–29. No bacteria.

July 3. Reinfection; 4 bacteria per field.

July 7–19. 1 bacterium in 20 fields; sometimes none.

July 23. Slight reinfection; 6 bacteria per field.

July 27. No bacteria; complete healing.



TEXT-FIG. 3. Experiment 3. Case 491. Effect of chloramine-T, 4 parts per 1,000, upon the asepsis of a wound.

Experiment 3. Case 491. Wound Kept Aseptic with Chloramine-T, 4 Parts per 1,000. Slight Reinfection.—Wound on inner side of right thigh.

Apr. 13, 1916. Wound is covered with necrotic tissue. Moderate infection. Sterilization with sodium hypochlorite.

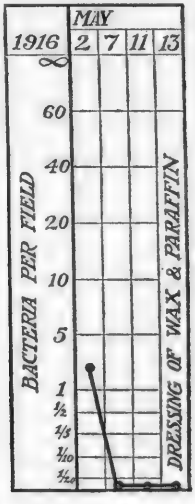
June 3. The wound is surgically sterile.

June 4. Wound is granulating and still shows small sections of necrotic aponeurosis. In these parts alone about 3 bacteria per field are found. The major portion of the wound is covered with surgically sterile granulations. Washing with neutral sodium oleate; dressing with 4 parts per 1,000 of chloramine-T.

June 5–15. The wound is dressed in the same manner and remains surgically sterile (Text-fig. 3).

July 9. Reinfection of wound; 8 to 10 bacteria per field. Dressing with chloramine-T, 10 parts per 1,000.

July 13. Wound is sterile.
July 14. Chloramine dressing, 4 parts per 1,000.
July 13–29. Wound has remained sterile.



TEXT-FIG. 4. Experiment 4. Case 450. Effect of chloramine-T, 4 parts per 1,000, upon the asepsis of a wound.

Experiment 4. Case 450. Sterilization of a Slightly Infected Wound and Preservation of Asepsis with Chloramine-T, 4 Parts per 1,000.—Wound on outer surface of right leg; sterilized with sodium hypochlorite.

May 2, 1916. The secretions contain 2 bacteria per field (Text-fig. 4).

May 3. Wound is washed with neutral sodium oleate; dressing with chloramine-T, 4 parts per 1,000.

May 7. All the bacteria have disappeared.

May 13. Grafts of skin are applied to the granulations and the wound is dressed with wax and paraffin.

Experiment 5. Case 327. Reinfection of a Sterile Wound Treated with Chloramine-T, 4 Parts per 1,000.—Wound in the antero-external region of the left thigh; sterilized with sodium hypochlorite and chloramine-T solution.

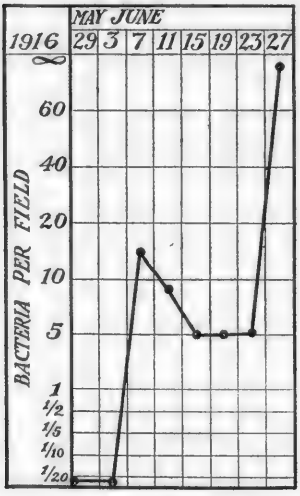
May 25, 1916. The wound, which is 25 sq. cm. in area, is surgically sterile. Dressing with chloramine-T, 4 parts per 1,000.

May 29. Wound sterile (Text-fig. 5); same dressing.

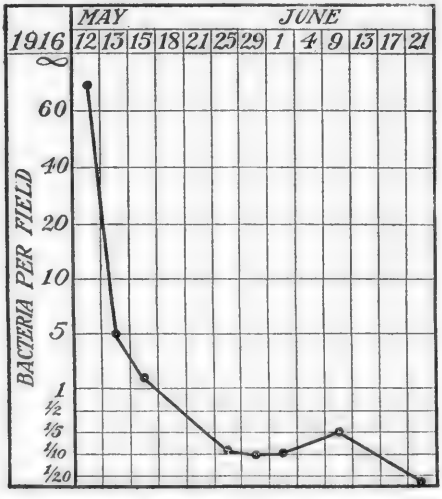
June 3. Wound sterile; same dressing.

June 7. Reinfection; same dressing.

June 27. Large number of bacteria.



TEXT-FIG. 5. Experiment 5. Case 327. Reinfection of an aseptic wound in spite of treatment with chloramine-T, 4 parts per 1,000.



TEXT-FIG. 6. Experiment 6. Case 445. Infection of cutaneous origin of a smooth and granulous wound in the foot. Sterilization with chloramine-T, 4 parts per 1,000.

Experiment 6. Case 445. Sterilization with Chloramine-T, 4 Parts per 1,000, of a Small, Slightly Infected Surface Wound.—Wound in the dorsal region of the foot.

May 12, 1916. Wound shows grayish granulations and the secretions contain approximately 100 bacteria per field (Text-fig. 6). Dressing of chloramine-T, 4 parts per 1,000.

May 29. Wound is surgically sterile and remains sterile through June 21, at which date complete healing is effected.

It has thus been shown that wounds in the fleshy regions could be maintained in a condition of surgical asepsis with sodium stearate containing 4 parts per 1,000 of chloramine-T. It was also possible to sterilize wounds presenting a slight infection of cutaneous origin. But in other cases the 4 parts per 1,000 of chloramine-T failed to maintain the asepsis. In Experiment 5 cutaneous reinfection developed in spite of the daily application of chloramine-T. The same occurred in Experiments 2 and 3, in which bacteria reappeared. It is probable, therefore, that the concentration of chloramine-T was too weak.

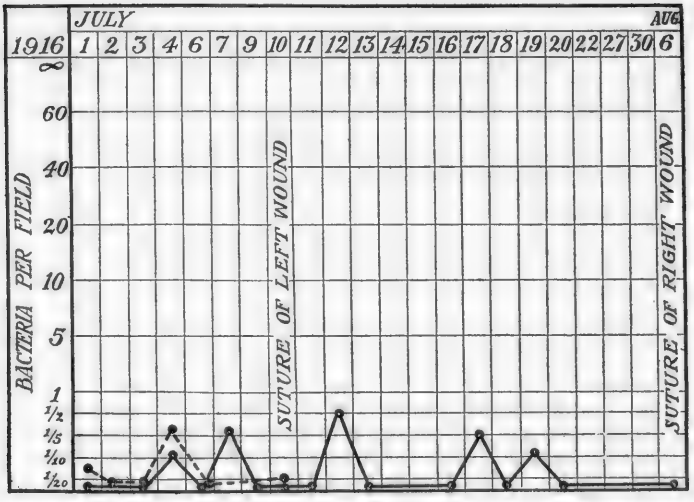
Effect of a Paste Containing 10 Parts per 1,000 of Chloramine-T upon the Bacteriological Condition of an Aseptic or Slightly Infected Wound.

Wounds, in some cases accompanied by fracture, were sterilized with Dakin's solution, and then dressed with chloramine-T, 10 parts per 1,000.

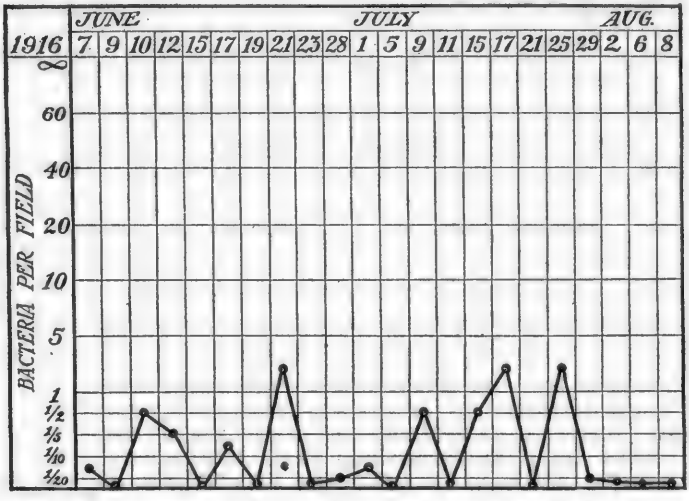
Experiment 7. Case 548. Preservation of Asepsis with Chloramine-T, 10 Parts per 1,000, of Two Penetrating Wounds in the Region of the Buttocks, Accompanied by Extensive Tissue Detachment.—Deep wounds in both buttocks caused by a projectile which entered the right thigh, came out between the buttocks in the anal region, and penetrated the left buttock, where it produced extensive muscular detachment. The large wounds and the intramuscular channels were sterilized with Dakin's hypochlorite solution.

July 1, 1916. The right wound has a surface area of 4 sq. cm., and the left of 10 sq. cm. These wounds communicate by means of deep anfractuous passages with the wounds located in the vicinity of the anus. Chloramine-T, 10 parts per 1,000, is carefully injected into the channels. This dressing is repeated daily.

July 6. The lower portion of the left wound has closed (Text-fig. 7). The deep channels healed spontaneously; the cavity separating the right wound from the anal region was kept in an aseptic condition by means of chloramine-T, and its sides grew together without the need of counter-incision.



TEXT-FIG. 7. Experiment 7. Case 548. Preservation of asepsis of two deep wounds in the region of the buttocks by the use of chloramine-T, 10 parts per 1,000. The solid line indicates the right wound; the broken line, the left wound.



TEXT-FIG. 8. Experiment 8. Case 516. Preservation of asepsis in an open fracture by means of chloramine paste, 7 and 10 parts per 1,000.

July 10. Closing of the left wound.

Aug. 6. The right wound is sutured. The curve indicated that the wounds remained aseptic during the treatment.

Experiment 8. Case 516. Maintenance of an Osseous Cavity in an Aseptic Condition with Chloramine-T, 7 and 10 Parts per 1,000.—May 17, 1916. Fracture of the upper part of the left tibia. Local sterilization with sodium hypochlorite.

June 4. At the level of the fracture there is a cavity the size of a small egg, containing grayish secretions. The opening of the cavity is at the base of a wound situated on the inner surface of the leg. The wound and cavity are surgically sterile. They are filled with chloramine-T paste, 5 parts per 1,000.

June 6. The paste has not been absorbed. Between the paste and the surface of the cavity is a fluid, transparent substance. Slight reinfection; 4 bacteria per field. Chloramine dressing, 7 parts per 1,000.

June 7. Wound is sterile (Text-fig. 8).

June 13. Wound is sterile. After the osseous cavity has been filled with chloramine paste a portion of the wound is sutured, but the loss of cutaneous substance effected at the time the wound occurred prevents its complete closure. The open part of the wound is covered with chloramine paste, 7 parts per 1,000. The wound remains aseptic.

July 10. Chloramine paste is removed from the osseous cavity. The walls of the cavity are seen to be covered with granulations. Without removing the paste which remains in the cavity, the latter is filled with chloramine paste, 10 parts per 1,000. The osseous cavity has remained completely aseptic. The slight reinfections shown by the curve exist only at the edges of the wound.

Aug. 22. The cavity has almost filled and is covered with epithelium.

Experiment 9. Case 625. Preservation of Asepsis in Five Flesh Wounds with Chloramine-T, 10 Parts per 1,000.—Numerous wounds in the left leg produced by bursting shells.

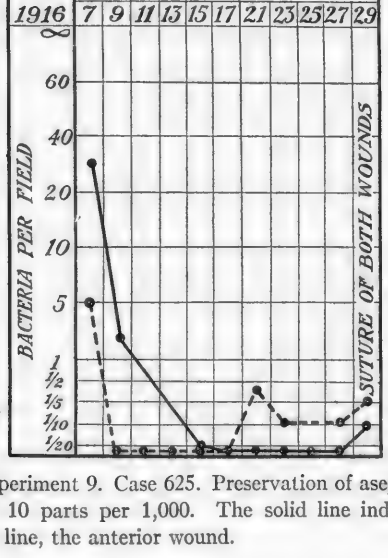
Sept. 5, 1916. Patient admitted to the hospital. The wounds are covered with pus and are treated with sodium hypochlorite.

Sept. 11. The pus has disappeared and the wounds are sterile (Text-fig. 9). Chloramine dressing, 10 parts per 1,000.

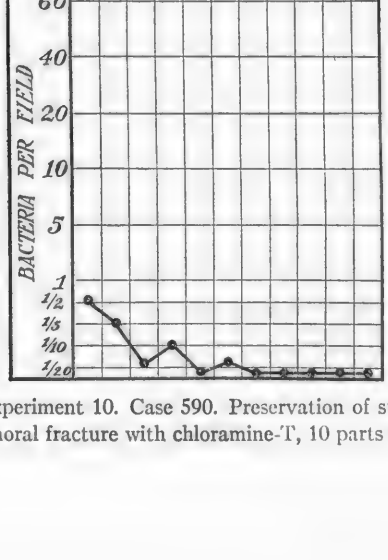
Sept. 13. Suture of three of the wounds; the other two are dressed with chloramine-T. They remain sterile through Sept. 29, when they are sutured.

Experiment 10. Case 590. Preservation of Asepsis in a Flesh Wound with Chloramine-T, 10 Parts per 1,000.—Wounds in the right thigh, accompanied by femoral fracture, produced by bursting shells. Treated with sodium hypochlorite.

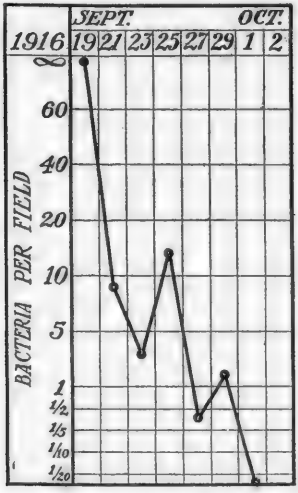
Sept. 11, 1916. The anterior wound is surgically aseptic (Text-fig. 10). Chloramine dressing. The wound is separate from the site of the fracture and remains sterile through Sept. 29 under chloramine dressing.



TEXT-FIG. 9. Experiment 9. Case 625. Preservation of asepsis of flesh wounds with chloramine-T, 10 parts per 1,000. The solid line indicates the posterior wound; the broken line, the anterior wound.



TEXT-FIG. 10. Experiment 10. Case 590. Preservation of sterility in a wound accompanied by femoral fracture with chloramine-T, 10 parts per 1,000.



TEXT-FIG. 11. Experiment 11. Case 620. Sterilization of a slightly infected wound with chloramine-T, 10 parts per 1,000.

Experiment 11. Case 620. Sterilization of a Slightly Infected Flesh Wound with Chloramine-T, 10 Parts per 1,000.—Numerous shell wounds. A large wound in the left buttock is treated with sodium hypochlorite. It is gradually cleansed.

Sept. 25, 1916. Wound contains 10 to 20 bacteria per field (Text-fig. 11). There are practically no secretions. Chloramine dressing, 10 parts per 1,000.

Sept. 27. Wound is surgically aseptic; same dressing.

Oct. 1. The bacteria have completely disappeared.

Experiment 12. Case 634. Reinfection Followed by Sterilization of Two Flesh Wounds with Chloramine-T, 10 Parts per 1,000.—Numerous shell wounds on the forehead, shoulder, and elbow. Disinfection of wounds with Dakin's hypochlorite solution.

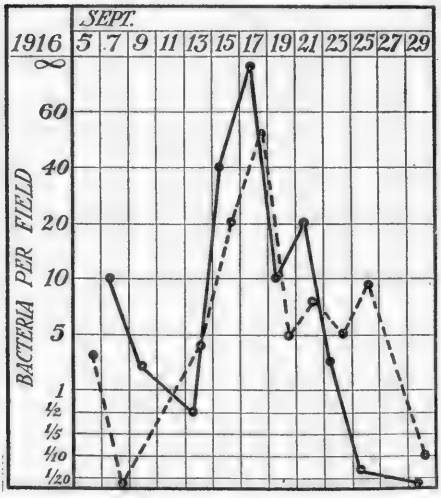
Sept. 11, 1916. The wounds on the forehead and left shoulder are almost sterile (Text-fig. 12). Application of chloramine paste and gauze dressing. The patient, who has also a severe fracture of the elbow and several other wounds, is very restless and the surface dressings become displaced.

Sept. 15 and 17. Reinfection from the skin. Considerable increase in the number of bacteria.

Sept. 17. Washing with neutral sodium oleate. The chloramine dressing is firmly fixed in place.

Sept. 19 and 21. Rapid diminution in the number of bacteria. Same dressing.

Sept. 29. Both wounds are sterile.



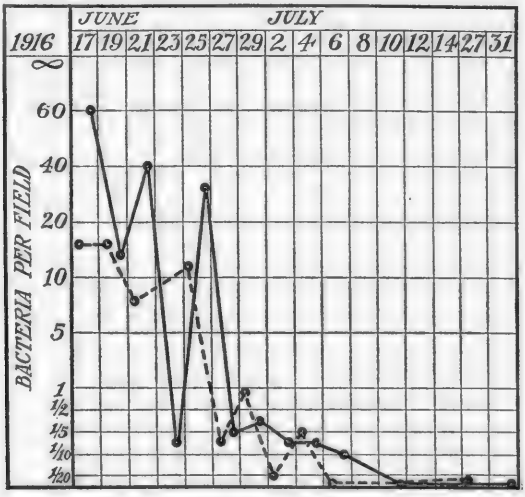
TEXT-FIG. 12. Experiment 12. Case 634. Reinfection of cutaneous origin, caused by shifting of dressing, and subsequent sterilization with 10 parts per 1,000 of chloramine-T. The solid line indicates the wound on the forehead; the broken line, the wound in the left shoulder.

The six experiments given above were selected from a series of sterile or slightly infected wounds treated with chloramine-T paste, 10 parts per 1,000. In every case the chloramine-T either maintained or produced the sterility of wounds infected from the skin. Nevertheless, this result can only be obtained if the dressings are made according to the technique described above. In Experiment 12, the dressings had not been firmly fixed at the surface of the wounds. The gauze shifted slightly and a cutaneous reinfection occurred. But sterilization was reestablished as soon as the appropriate technique was applied.

Sterilization of a Wound with Chloramine-T, 10 Parts per 1,000.

The above experiments and many others having shown that chloramine-T, 10 parts per 1,000, preserves a wound in an aseptic condition for several days and weeks, we next endeavored to ascertain whether it would also effect the sterilization of an infected wound.

The first of these experiments was made with surface wounds showing no necrotic tissue and slight infection. Later they were applied to wounds accompanied by a fracture.



TEXT-FIG. 13. Experiment 13. Case 519. Sterilization of two surface wounds of the left thigh with chloramine-T, 10 parts per 1,000. The solid line indicates the inside wound; the broken line, the posterior wound.

Experiment 13. Case 519. Sterilization of Two Flesh Wounds with Chloramine-T, 10 Parts per 1,000.—Two wounds of the thigh sterilized with sodium hypochlorite; subsequent reinfection. The wound on the inner side measures 24 sq. cm.; that towards the back, 19 sq. cm.

June 25, 1916. Both wounds are covered with smooth red granulations. No necrotic tissue; small amount of secretion; slight infection. Cocci, diplococci, and bacilli appear on the inside wound in the proportion of 30 per field, and 12 per field in the posterior wound (Text-fig. 13). Application of chloramine paste, 7 parts per 1,000.

June 26. Inside wound shows cocci, diplococci, and streptococci averaging 10 to 15 per field. In the posterior wound there are masses of cocci—6 to 8 per field. Washing with neutral sodium oleate; dressing with chloramine paste, 7 parts per 1,000.

June 27. Inside wound contains 1 coccus in 5 fields; posterior wound contains 1 coccus in every 7 fields.

June 30. Chloramine dressing, 10 parts per 1,000.

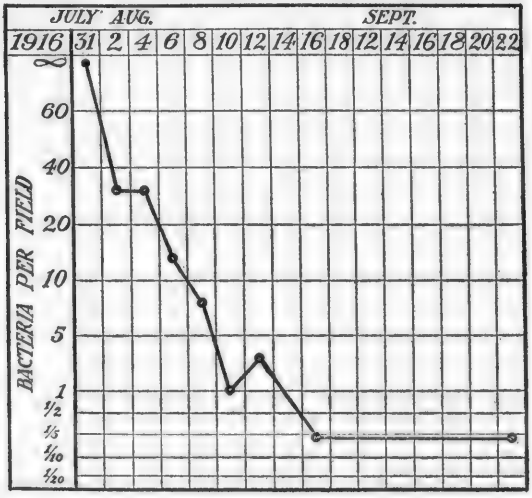
July 1. Inner wound shows 1 coccus in 8 fields; posterior wound shows 1 coccus in 8 fields. Same dressing.

July 4. Few bacteria. From this time on the wounds remain sterile.

July 27. The posterior wound has healed.

July 31. The inside wound has healed.

This experiment shows that a wound covered with smooth granulations, devoid of necrotic tissue, with a slight quantity of secretion, and a varied but sparse bacterial flora, may be completely sterilized with chloramine-T paste. The experiment was repeated with a large number of wounds and always produced identical results. Thereafter the same treatment was applied to more heavily infected wounds which still contained some necrotic tissue.



TEXT-FIG. 14. Experiment 14. Case 591. Sterilization of a seton wound in the sole of the foot with chloramine-T, 10 parts per 1,000.

Experiment 14. Case 591. Sterilization of a Seton Wound in the Sole of the Foot with Chloramine-T, 10 Parts per 1,000.—Seton wound in the sole of the foot produced by a fragment of a torpedo on July 24.

July 31, 1916. The wound is very much inflamed and infected. Large number of bacteria (Text-fig. 14). Treatment with Dakin's hypochlorite solution.

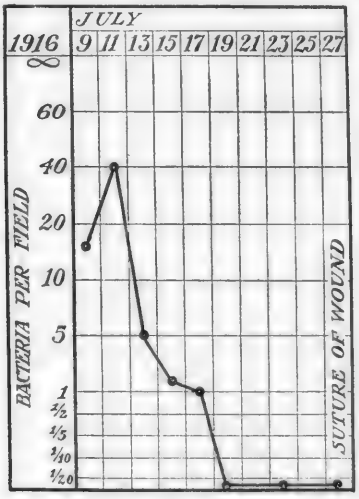
Aug. 3. Sole of the foot still infected. At the surface a small amount of necrotic tissue remains. Radiological examination of the wall of the wound shows the presence of nine minute fragments which cannot be removed owing to their small size. Injection of chloramine paste into the channel and chloramine dressing, 10 parts per 1,000.

Aug. 4. The wound still contains 30 bacteria per field.

Aug. 6 and 8. The wound is becoming sterile.

Aug. 10. The wound now contains only 1 bacterium per field.

Aug. 16. The wound has become surgically sterile. It remains in this condition through Sept. 22, when complete sterilization is effected.



TEXT-FIG. 15. Experiment 15. Case 562. Sterilization with chloramine-T, 10 parts per 1,000, of a slightly infected wound in the right buttock, accompanied by fracture of the ilium.

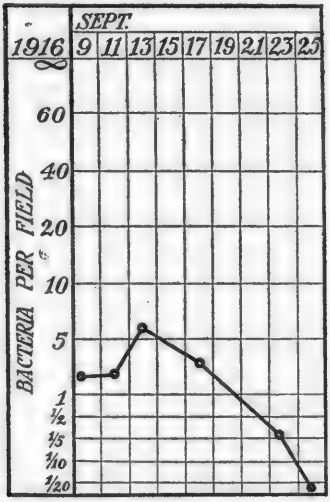
Experiment 15. Case 562. Sterilization of a Wound Accompanied by Fracture with Chloramine-T, 10 Parts per 1,000.—July 4, 1916. Large shell wound in the upper part of the right buttock and fracture of the ilium. Wound is first treated with Dakin's hypochlorite solution.

July 9. The surface of the wound is cleansed. The secretions average 15 bacteria per field (Text-fig. 15). Dressing with chloramine paste, 10 parts per 1,000.

July 10. Wound is covered throughout with red granulations; only at isolated points is it still grayish in color. Chloramine dressing, 10 parts per 1,000.

July 13. 5 bacteria per field.

July 17. The grayish spots have disappeared. About 1 bacterium per field.
July 20. Wound is sterile.
July 27. Wound is sutured.



TEXT-FIG. 16. Experiment 16. Case 646. Sterilization with 10 parts per 1,000 of chloramine-T of a deep, slightly infected wound.

Experiment 16. Case 646. Sterilization of a Slightly Infected Flesh Wound with Chloramine-T, 10 Parts per 1,000.—Sept. 8, 1916. Numerous suppurating and severely infected wounds on the left leg. Two of the wounds are treated for 48 hours with Dakin's hypochlorite solution.

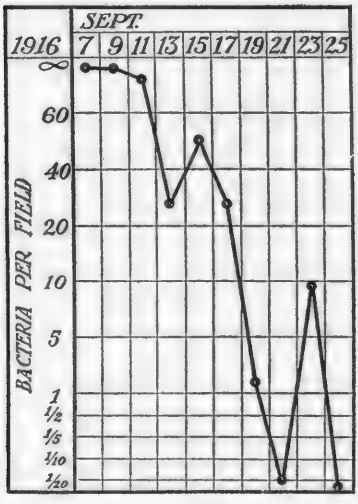
Sept. 11. The inside wound is still infected (Text-fig. 16). It is deep, and the calf is still inflamed. Chloramine dressing, 10 parts per 1,000.

Sept. 23. Wound is surgically sterile.

Experiment 17. Case 441. Sterilization of a Small, Severely Infected Wound with Chloramine-T, 10 Parts per 1,000.—Sept. 4, 1916. Removal by a bursting shell of the fourth and fifth fingers of the left hand, and of the greater part of the fifth metacarpal.

Sept. 6. Suppurating wound, severely infected and painful, containing necrotic tissue and large numbers of bacteria. Treatment for 5 days with Dakin's hypochlorite solution.

Sept. 11. The wound still contains large numbers of bacteria in the necrotic parts (Text-fig. 17). Red granulations have appeared. Dressing with chloramine paste, 10 parts per 1,000.



TEXT-FIG. 17. Experiment 17. Case 441. Sterilization of a severely infected wound containing a small quantity of necrotic tissue with chloramine-T, 10 parts per 1,000.

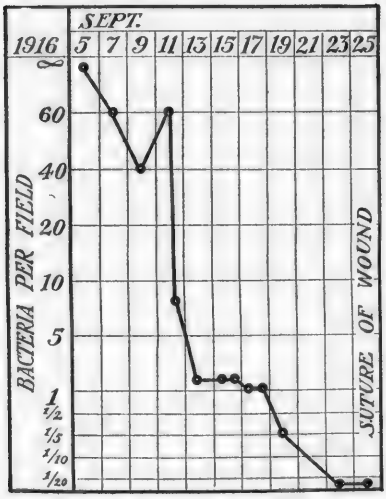
Sept. 13. The wound contains 30 bacteria per field. Same dressing.
Sept. 19. Wound is almost sterile; contains 1 bacterium per field.
Sept. 21. A few bacteria are found on a small fragment of necrotic aponeurosis.
Sept. 25. Wound is sterile.

Experiment 18. Case 626. Sterilization with Chloramine-T, 10 and 15 Parts per 1,000, of a Large, Severely Infected Wound, Accompanied by Fracture.—Extensive wound, more than half the thickness of the forearm in depth, with fracture of the radius and cubitus.

Sept. 5, 1916. The wound, which is 9 days old, appears grayish in color. The muscular and osseous surfaces have been evened, but they are still covered with necrotic tissue and blue pus. The limb is swollen and painful. Chloramine dressing, 10 parts per 1,000.

Sept. 7. The blue pus has almost completely disappeared. At the surface and bottom of the wound some necrotic tissue remains. Application of chloramine paste, 15 parts per 1,000, at the level of the osseous extremities. The rest of the wound is covered with chloramine-T, 10 parts per 1,000.

Sept. 13. The wound now contains 1 bacterium per field (Text-fig. 18). The granulations of the section of the wound treated with chloramine paste, 15 parts per 1,000, have assumed a deep red color. The necrotic tissue has completely disappeared.



TEXT-FIG. 18. Experiment 18. Case 626. Sterilization with 10 and 15 parts per 1,000 of chloramine-T of a wide, severely infected wound of the forearm, accompanied by fracture of the upper part of both bones, and with pyocyanic infection and necrotic tissue.

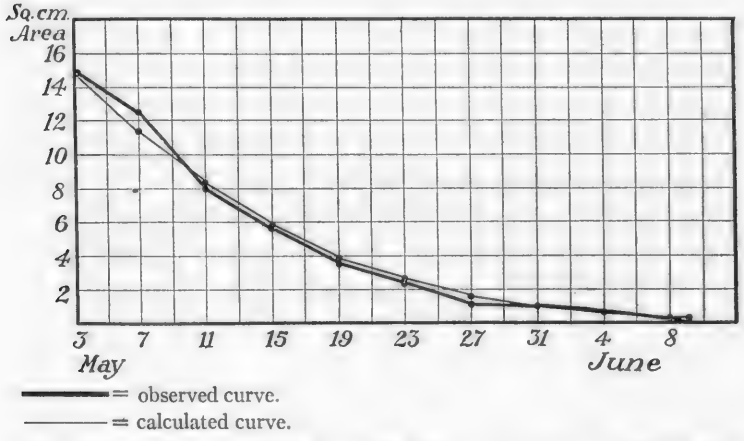
Sept. 19. Wound is surgically sterile.
Sept. 23. The bacteria have disappeared from the films.
Sept. 25. Wound is sutured.

The wounds in the above experiments were in different stages of infection. Some were simple flesh wounds unaccompanied by necrotic tissue and with but slight suppuration. Others showed necrotic tissue and greater suppuration, while in a third group cases of severe local infection were accompanied by fracture. The wound in Experiment 18 was complicated by fracture of the radius and ulna and showed abundant suppuration. Moreover, it contained a large number of *pyocyaneus* bacilli. All these wounds were easily sterilized with chloramine-T paste, 10 or 15 parts per 1,000. In 48 hours the blue pus had almost completely disappeared from Wound 18. But sterilization took place more slowly than would have been the case if instillations of sodium hypochlorite had been used. These results were obtained with a paste containing 10 to 15 parts per 1,000 of

chloramine-T. The 15 parts per 1,000 of chloramine-T caused slight irritation to the skin and congested the granulations. Apparently the concentration of chloramine-T must not exceed 10 parts per 1,000.

Effect of Chloramine Paste on the Rate of Cicatrization.

In the following experiments an attempt was made to determine whether chloramine-T, when used in a concentration of 4 and of 10 parts per 1,000, has any effect upon the process of cicatrization. The chloramine paste was applied to wounds whose surface was measured every 4 days, and whose healing curve had been calculated according to the formula of du Noüy. By comparing the curves as calculated and observed we were able to determine whether the action of the chloramine paste retarded the process of cicatrization. Experiments were made with surface wounds treated with chloramine-T in a concentration of 4 and 10 parts per 1,000. The results were approximately identical in all cases. Only two typical experiments will be described.



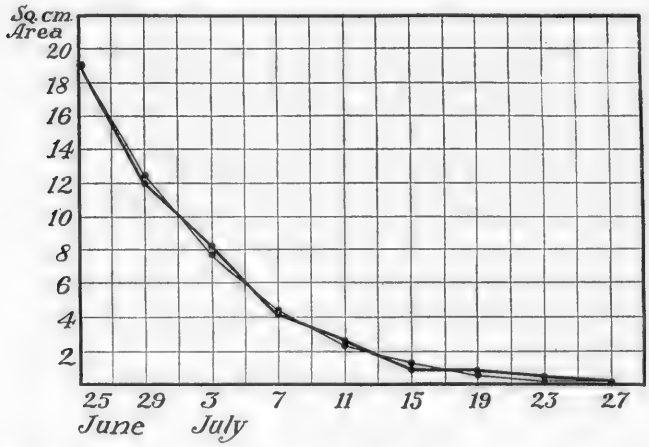
TEXT-FIG. 19. Experiment 19. Case 450. Effect of 4 parts per 1,000 of chloramine upon the rate of cicatrization of a wound. Application of chloramine paste on May 3 to 13.

Experiment 19. Case 450. Effect of Chloramine-T, 4 Parts per 1,000, on the Cicatrization of an Aseptic Wound.—Granulous wound.

May 3, 1916. Wound almost sterile and contains only a few bacteria. The surface measures 15 sq. cm. The cicatrization curve, calculated according to the formula of du Noüy, shows that the healing process should be accomplished on June 8. Chloramine dressing, 4 parts per 1,000.

May 7. The wound is sterile; same dressing. From this time on the cicatrization curve descended and caught up with the calculated curve (Text-fig. 19).

From May 11 to 13, at which time wax was substituted for the chloramine-T, the curve followed the calculated curve.



TEXT-FIG. 20. Experiment 20. Case 519. Effect of chloramine-T, 7 and 10 parts per 1,000, upon the rate of cicatrization of a wound. The wound was dressed from June 27 to 30 with chloramine-T, 7 parts per 1,000, and from June 30 to July 27 with chloramine-T, 10 parts per 1,000.

Experiment 20. Case 519. Effect of Chloramine-T, 7 and 10 Parts per 1,000, upon the Cicatrization of an Aseptic Wound.—Granulating wound at the back of the thigh.

June 25, 1916. Superficial measurement, 19 sq. cm. The secretions contain 10 bacteria per field. The course of cicatrization is calculated according to the formula of du Noüy.

June 27. The wound is surgically aseptic. Chloramine dressing, 7 parts per 1,000, is used until June 30.

June 30 to July 27. Chloramine dressing, 10 parts per 1,000. The curve coincided almost exactly with the calculated curve; nevertheless, after July 19 slight retardation of the healing process apparently occurred (Text-fig. 20).

The above experiments, as well as others that are analogous, show that sodium stearate containing from 4 to 10 parts per 1,000 of chloramine-T does not retard the process of cicatrization to an appreciable extent. In Experiment 20 during the first 25 days the observed curve coincided with the calculated curve, after which the rate of healing became slightly reduced.

SUMMARY.

Sodium stearate has no effect upon the bacteriological condition of a wound, but the addition of 4 parts per 1,000 of chloramine-T renders it antiseptic. Experiment 1 enabled us to compare the action of sodium stearate alone with that of sodium stearate containing 4 parts per 1,000 of chloramine-T. Wounds which had been previously sterilized could be maintained in an aseptic condition by 4 parts per 1,000 of chloramine-T, although in some cases reinfection occurred (Experiments 2, 3, and 5). For this reason the concentration of chloramine-T was increased.

Surface wounds, deep-seated wounds, and osseous cavities, which had previously been either completely or almost completely sterilized, could be maintained for days and even weeks in a condition of surgical asepsis by the use of a paste containing 7 and 10 parts per 1,000 of chloramine-T. Experiments 7, 8, 9, 10, and 12 are examples of this. Slightly infected wounds (Experiments 9, 11, and 12) were sterilized in the same manner.

Next, it was attempted to sterilize wounds which were suppurating and more or less infected, and in some cases accompanied by fracture. This attempt was probably successful because the wounds used for the experiments showed but slight quantities of secretions and only a shallow layer of necrotic tissue. It is useless to attempt to sterilize severely infected wounds with a paste, for the volume of chloramine-T that can be applied is too limited. A large volume of an active substance is required to sterilize a wound which secretes great quantities of pus, for owing, on the one hand, to the dilution of this substance with the secretions, and, on the other, to its combination with the proteins contained in the pus, the concentration of the antiseptic is rapidly diminished. For these reasons it is essential that the antiseptic solu-

tion should be constantly renewed, so that the concentration may be sufficiently strong to effect the destruction of the bacteria. Therefore, the chloramine-T paste cannot sterilize a severely infected wound.

The concentration of the active substance contained in a paste must at the same time be sufficiently weak to be innocuous to the tissues. We have seen that it should not exceed 15 parts per 1,000. Thus, it is evident that if the secretions from the wounds are abundant, the substance could exert its action upon the microorganisms for the space of only a few hours. For this reason the chloramine paste should only be applied under the conditions specified in our experiments; that is, in connection with moderately infected wounds which have been carefully washed with sodium oleate, and possess but slight quantities of secretion. Under these conditions, the chloramine paste effects the complete disappearance of the bacteria and maintains the sterility thus secured for as long a time as may be wished. If the technique followed in the dressing is not exactly as above described, reinfection will occur. If applied in this manner the chloramine paste is not injurious to the tissues, for the cicatrization curves of the wounds thus treated show but slight modification from the calculated curves.

Chloramine paste makes it possible, therefore, to keep wounds sufficiently free from microorganisms so that the effect of substances which are believed to influence cicatrization can be studied.

CONCLUSIONS.

1. Under the conditions of our experiments chloramine paste maintains the asepsis of a wound already sterile, and sterilizes an infected wound.

2. Under the same conditions chloramine paste causes no apparent modification of the cicatrization curve of an aseptic wound.

TOXIN AND ANTITOXIN OF AND PROTECTIVE INOCULATION AGAINST BACILLUS WELCHII.

By CARROLL G. BULL, M.D., and IDA W. PRITCHETT.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, April 15, 1917.)

This study of the pathogenicity of infection by the group of *Bacillus welchii* has followed from several fortuitous circumstances. First, there was the insistent problem, only partially solved by the improvement in the antiseptic treatment of wounds, of gas bacillus infection following shell and bullet wounds everywhere in the war; second, there were available to us several cultures of *Bacillus welchii* isolated during the summer of 1916 on the western battle front by Dr. Simonds, who kindly presented them to the Institute; and finally and especially, Dr. Flexner's wish that with these cultures the subject of gas bacillus infection of the pigeon which he had observed many years before at the Johns Hopkins Hospital should be reinvestigated, as, in his opinion, the process in that species of animal epitomized the pathologic effects occurring in gas gangrene in man, and because he believed that a better understanding of the one condition would serve to explain many still obscure points in the other.

It will be of interest in this connection to review briefly certain facts concerning gas bacillus infection in the pigeon, since the condition is one little known to pathologists and bacteriologists. The classical article by Welch and Nuttall[1] on the gas bacillus appeared in 1892. It was followed by a paper on gas bacillus infection in man by Welch and Flexner[2] in 1896. The latter article was incomplete,[3] and the concluding part which was to appear in the next number was never published. The second paper was to deal more particularly with experimental gas bacillus infection in animals—in the guinea

[1] Welch, W. H., and Nuttall, G. H. F., *Bull. Johns Hopkins Hosp.*, 1892, iii, 81.

[2] Welch, W. H., and Flexner, S., *J. Exp. Med.*, 1896, i, 24.

[3] Personal communication from Dr. Flexner.

pig and pigeon particularly. Since the pigeon had proved to be highly subject to infection and to respond with characteristic pathologic reactions, that animal came to be employed by the laboratory staff in the more or less routine study of the gas bacillus. But because of the circumstances stated, no full and sufficient description of the local lesions in the pigeon, which in disorganizing effect are comparable with the destructive lesions sometimes present in man, came to be published until some years later, when, at Dr. Flexner's suggestion, Dr. Herter,[4] then engaged in the study of the *Bacillus welchii* group of bacteria occurring in the alimentary tract, employed this animal for inoculation. The lesions as described by Herter agree closely with those present in our pigeons inoculated with cultures of the bacilli.

Sources and Nature of Cultures.

The main part of our experiments has been made with five strains of *Bacillus welchii*, of which four were obtained through the kindness of Dr. Simonds. The fifth was isolated by us from a piece of clothing which had long been worn. The history of the Simonds cultures follows. The tests given were made by him.

Strain 365 a.—Isolated, Aug. 13, 1916, from scrapings from a bullet wound of the thigh, which showed a moderately severe gaseous gangrene. Its virulence for laboratory animals had not been tested.

Strain 386 cd.—Isolated, Sept. 9, 1916, from a fragment of shell with adherent bits of clothing, removed from a wound of the thigh. The patient did not develop gaseous gangrene. The organism had not been pathogenic for guinea pigs.

Strain 617 d.—Isolated, Aug. 27, 1916, from a case of violent gaseous gangrene following a bullet wound of the thigh with injury to and subsequent ligature of the large vessels of the leg. The limb was amputated. Although the stump was gaseous, the patient recovered. This strain is very pathogenic for guinea pigs, producing typical lesions and killing the animal in less than 24 hours after subcutaneous injection of 0.5 cc. of a 24 hour dextrose broth culture.

Strain 669 b.—Isolated, Aug. 21, 1916, from a case of gaseous gangrene following a bullet wound which caused shattering of the lower end of the femur. The leg was amputated; the patient recovered. Injection of 0.5 cc. of a 24 hour dextrose broth culture subcutaneously into a guinea pig was followed by a local-

[4] Herter, C. A., Bacterial infections of the digestive tract, New York, 1907, 198.

ized gaseous gangrene and sloughing of the skin and subcutaneous tissues. The animal died 2 weeks after the injection. No gas bacilli were found at autopsy in films from tissues adjacent to the slough.

The history of our own strain is as follows: A piece of cloth from the lining of an old overcoat was thrust with tissue forceps deep into the right breast muscle of an anesthetized pigeon. The next morning the wounded muscle was greatly swollen, and crepitation was present. Pressure near the wound forced out gas bubbles which ignited with a snap. The pigeon was drooping and died at 2.30 p.m.

Autopsy.—The skin over the right side was edematous and covered with blebs. There was a reddish brown gelatinous exudate in the subcutaneous tissues of both groins and extending over the right pectoral muscles. A film preparation made from this exudate contained a few plump Gram-positive bacilli. The inoculated muscle was edematous and necrotic, most pronounced about the cloth, but extending along the muscle sheaths to the insertion of the fibers. Films from the necrotic muscle contained many plump Gram-positive bacilli and a few Gram-positive diplococci and Gram-negative bacilli.

Tubes of recently boiled litmus milk, blood agar, and blood bouillon were inoculated with the subcutaneous exudate and necrotic muscle. After 24 hours' incubation, the milk tubes showed the so called "stormy fermentation," and those inoculated with the subcutaneous exudate contained a plump Gram-positive bacillus in pure culture. The tubes from the necrotic muscle contained a similar bacillus, together with Gram-positive cocci in pairs and short chains and a Gram-negative bacillus. The blood media inoculated with the subcutaneous exudate remained sterile, while those of the necrotic muscle yielded cocci and Gram-negative bacilli only, the latter proving to be a variety of *Bacillus coli.* We shall call the culture of the Gram-positive bacillus, to be described more fully later, P-50.

The five cultures of Gram-positive bacilli enumerated have been tested for motility, spore formation, quantitative acid and gas production, liquefying action on gelatin at 22° and 37°C., pathogenicity for guinea pigs, rabbits, and pigeons, and still other properties. For example, the several strains have been tested for agglutination and lysis in normal rabbit and guinea pig sera, for hemolysins, for gas production in rabbits after the method of Welch and Nuttall, and for agglutination in artificially produced immune sera. As far as these tests are indicative, they place all five cultures among the group of *Bacillus welchii;* so far as they relate to specific properties, *e.g.*,

specific and cross agglutination, they indicate certain differences among them such as have commonly been observed among members of the group.

Pathologic Effect in Animals.

The feature of most importance among the properties just given which define this group of bacilli is pathogenic effect. This is true in the first place because of the similarity of certain of the lesions in animals to those arising in gaseous gangrene in man; and next and especially, because the investigation of the manner in which the lesions arise in animals led us to the discovery of the conditions under which a highly potent soluble toxic agent is regularly produced by the bacilli on which their poisonous or lethal action chiefly if not wholly depends.

In the past, the laboratory animal usually employed to test pathogenicity of *Bacillus welchii* has been the guinea pig. Many more cultures produce mild local infections, from which recovery takes place, than severer ones which are fatal. But the subcutaneous or intramuscular injection of cultures gives rise to local swelling, gas production, liquefactive necrosis of the involved muscle and skin, and, if the animal survives, eventual sloughing and cicatrization.

The rabbit is more resistant to local infection than is the guinea pig, but nevertheless lesions can be produced by active cultures which in general resemble those of the guinea pig. The rabbit reacts either to more highly virulent cultures or to larger doses of less effective ones. In the case of the former, death may result. But in both the rabbit and the guinea pig general blood invasion, even when the course of the infection is lethal, either does not take place at all or so few bacilli enter the blood that the cause of death cannot be attributed to a septicemia.

The peculiar susceptibility of the pigeon to infection with *Bacillus welchii* has not been utilized to the degree deserved. For in no other laboratory animal is infection produced so readily and with such a wide number of cultures, and in no other does the pathologic process proceed so swiftly and characteristically and with effects so nearly resembling the condition of gaseous gangrene in man. Probably this lack of use of the pigeon is to be ascribed to the rather brief refer-

ences made to the subject in the literature, which is almost confined to a few summary statements emanating from the Johns Hopkins Hospital at about the period already referred to. Dr. Flexner himself employed mainly blood containing large numbers of the bacilli for inoculating pigeons. In some instances the blood was taken post mortem from human cases, and in others from rabbits injected, killed, and incubated by the Welch-Nuttall method. Inoculation from pigeon to pigeon was also carried out. The animals succumbed at periods ranging from 5 to 24 hours. The autopsies revealed lesions precisely like those already described (page 121), except that the injected blood would often remain to tinge the tissues. The bacilli were very numerous in the disorganized muscle, fewer in the gelatinous exudate, and very few in or absent from the heart's blood.

The five cultures were pathogenic for pigeons, although not equally so. Simonds' Culture 617 d proved most virulent, not only for pigeons, but for rabbits and guinea pigs also. Doses of 0.01 to 0.05 cc. of a 20 hour glucose broth culture injected directly into the breast muscles were invariably fatal to pigeons in 6 to 20 hours. 0.0005 cc. of such a fluid culture put upon a bit of gauze and introduced into the muscles usually but not invariably produced fatal infection. Of Cultures 365 a and 669 b approximately 0.1 cc. of a glucose broth culture was the minimal lethal dose; while the dose of Cultures 386 cd and P-50 required to produce corresponding effects was 0.2 to 0.3 cc. The minimal lethal doses for guinea pigs and rabbits were several times those used for the pigeons.

Cause of Death in Bacillus welchii Infection.

In man, infection with *Bacillus welchii* tends to be a local process, even when severe, and invasion of the general blood occurs if at all only during the death agony or post mortem. In a small number of instances in man general infection seems to have played an important part in causing or hastening death.[5] But as these cases are the exception, even when death occurs, in man as well as in the pigeon, rabbit, and guinea pig, it may be assumed that soluble chemical substances entering the circulation from the local lesion bring about

[5] Thaon, P., *Compt. rend. Soc. biol.*, 1908, lxiv, 863.

the severe symptoms and the fatal termination. The question at once arising from this general consideration relates to the probable nature of the poisonous bodies. Several possibilities present themselves: They may arise from the bacilli, they may be yielded by the disorganizing tissues, or they may be of the nature of acids which disturb profoundly the hydrogen ion concentration of the body fluids. The protocols which follow are given to show the manner in which death may be produced in rabbits and pigeons under circumstances in which the blood remains wholly or practically free of the bacilli.

Experiment 1.—Pigeon. 11 a.m. 0.2 cc. of a glucose broth culture of Culture 617 d was injected into the breast muscle. 6.30 p.m. Animal died. Immediate autopsy. Usual gaseous, gelatinous, and necrotic local lesions containing myriads of bacilli. Films from the heart's blood and peritoneal fluid showed no bacilli, although a few were present, as a glucose broth culture was positive.

Experiment 2.—Pigeon. 11 a.m. 2 cc. of broth culture of Culture 617 d injected into breast muscles. 2.30 p.m. Animal died, having survived $3\frac{1}{2}$ hours. Local lesions similar to the preceding experiment, but the films from heart's blood contained a few bacilli.

These experiments were varied and repeated with different doses of the culture, but in no instance did a severe septicemia develop.

Experiment 3.—Rabbit. 11 a.m. Sedimented bacilli from 10 cc. of a 24 hour glucose broth culture of Culture 365 a were injected into the ear vein. 12 m. Films from the heart's blood showed a few bacilli, and the culture was positive. 9.30 p.m. Animal stuporous and breathing heavily. 10 p.m. Films and cultures from heart's blood negative. 10.20 p.m. Died. Immediate autopsy showed a dark and large spleen, but blood intact and serum clear. Films and cultures yielded no bacilli from the blood but some from the spleen and liver.

Experiment 4.—Rabbit. Mar. 14, 1917. 11 a.m. Sedimented bacilli from 2 cc. of broth culture No. 617 d given intravenously. Mar. 19, 8 a.m. Dead. Autopsy showed bloody fluid in peritoneum and small amount of clear fluid in pleura. The former contained many, the latter few bacilli; the heart's blood contained none.

It is apparent from these experiments that the death of the animals—pigeons and rabbits—is not closely bound up with the multiplication of the bacilli in the general circulation. The rabbit experiments indicate that the bacilli have no power to remain in the blood stream, and even when diminishing in numbers in the internal

organs still exert poisonous effects. Hence it may be concluded that poisons are liberated from the bacilli, but whether merely secreted or only yielded upon disintegration is not indicated by these tests. The next step therefore was to look for soluble toxic substances in the fluid cultures. For this purpose 24 hour glucose broth growths were employed.

Experiment 5.—(*a*) Rabbit; weight 1,950 gm. 8 cc. of Culture 669 b were injected intravenously. Immediately following the injection the animal became greatly excited, respiration became rapid, prostration followed, and death occurred in 7 minutes from respiratory failure.

(*b*) Rabbit; weight 1,700 gm. 9 cc. of Culture 617 d similarly injected. 14 minutes later this animal suddenly became excited, jumped from the basket, and died 7 minutes later of respiratory failure.

(*c*) Rabbit; weight 1,800 gm. 10 cc. of Culture 365 a intravenously injected. Died within 6 minutes under similar circumstances to those in (*a*) and (*b*).

In other words, an acutely fatal effect can be produced from large quantities of a broth culture injected intravenously. Less quantities (1 to 2 cc. per kilo) produce no immediate symptoms but cause death in from 6 to 24 hours. Differences in virulence only appear from the smaller doses and at once distinguish Culture 617 d as the most active. The massive doses of cultures exerted an injurious effect on the red blood corpuscles, which are destroyed in large numbers. On the other hand, the bacilli do not tend to agglutinate rapidly in the blood stream. By performing *intra vitam* agglutination tests, it was found that Culture 386 cd alone became rapidly agglutinated. This one was the least active, requiring the largest dose to cause acute death. The blood became free of the other cultures only after 4 to 8 hours. It appears then that bacillary embolism is not the probable cause of the acute lethal effects.

The toxicity of the fluid portion of the broth cultures was investigated. Since in these tests centrifugation alone was employed, pigeons could not be used, as the small number of bacilli remaining was sufficient to cause infection; hence rabbits were again injected. We may mention here that the injection of 2 to 4 cc. of the centrifuged fluid subcutaneously or intramuscularly into rabbits would sometimes lead to severe local and fatal infection. The fluid therefore possessed aggressive activity.

Experiment 6.—(*a*) Rabbit; weight 1,475 gm. Injected 10 cc. of supernatant fluid of glucose broth culture No. 617 d intravenously. Immediate collapse, air hunger, and death in 2 minutes. The red corpuscles were largely disorganized. The serum was reddish brown, and the remaining corpuscles appeared as mere shadows.

(*b*) Rabbit; weight 1,400 gm. Before injection the red corpuscles numbered 5,600,000 and the white 8,700 per c.mm. 12 m. 4 cc. of supernatant fluid of Culture 617 d were injected intravenously. 20 minutes later breathing was rapid and labored; the animal was very weak, and the red cells had fallen to 80,000 per c.mm. The white cells were unchanged. 1.20 p.m. Animal prostrate, the red cell count 84,000, and the white cells 8,300 per c.mm. 10 minutes later respiration ceased. The kidneys were dark brown in color, the bladder contained dark brown urine, and the few remaining red corpuscles were shadowy.

(*c*) Rabbit; weight 1,375 gm. Red cells 5,250,000 per c.mm. 11.50 a.m. Received 3 cc. of supernatant fluid, Culture 617 d. 12.15 p.m. Respiration accelerated, red cells 1,600,000. 3 p.m. Red cells 1,650,000. Died in night. The urine found was dark brown in color. Kidneys chocolate colored.

It would appear to be shown by these experiments that the acutely fatal effects of massive doses of the broth cultures as such or when separated in large part from the bacilli themselves are due to some body causing rapid and extensive blood destruction. Whether any other factor plays a part these experiments do not determine.

The next experiments were devised to rule out the factor of acidity. The different supernatant fluids exhibited acidities ranging from 2.5 to 4.5 per cent in terms of normal sodium hydroxide, with phenolphthalein as indicator. References in the literature point to the acid and especially the butyric acid content of cultures as responsible for the toxic effects. Two sets of tests were made (1) by neutralizing the broth with sodium hydroxide and (2) by comparing the acidity with the toxicity of different fluids.

Experiment 7.—Rabbit; weight 1,575 gm. Red cells 5,450,000. 12 m. 5 cc. of supernatant fluid of Culture 617 d neutralized with sodium hydroxide were injected intravenously. 12.35 p.m. Red cells 1,600,000. 1.50 p.m. Red cells 800,000. Respiration rapid, animal tires easily. 3 p.m. Red cells 600,000. Animal died during night. The autopsy showed the dark urine and chocolate colored kidneys as described above.

The exclusion of the acidity in the fluid may diminish somewhat the intensity of the blood destruction but does not remove it or

prevent the fatal issue. Experiments similar to this were made a number of times with consistent results. Moreover, comparison of toxic action and the degree of acidity was made, from which it was seen that acidity and lethal effects do not proceed hand in hand.

Experiment 8.—(*a*) Rabbit; weight 1,400 gm. Red cells 5,700,000 per c.mm. 2.45 p.m. 8 cc. of supernatant fluid of Culture 617 d, having an acidity of 1.9 per cent normal sodium hydroxide, were given intravenously. 4.45 p.m. Red cells 1,240,000. 5 p.m. Animal breathing rapidly; very weak. Died during night. Usual autopsy findings.

(*b*) Rabbit; weight 1,600 gm. 2 p.m. 10 cc. of supernatant fluid of Culture 386 cd, having an acidity of 4.5 per cent normal alkali, were injected intravenously. No symptoms appeared, and the red corpuscles were only slightly reduced in number. The animal still lives.

These experiments show that the acidity is not the main factor in causing either blood destruction or the fatal effects, and they are supported by tests on pigeons, examples of which follow; they indicate also that *Bacillus welchii* produces, in the test-tube at least, an active hemolysin.

Experiment 9.—(*a*) Pigeon. Red cells 4,645,000 per c.mm. 9.50 a.m. 0.5 cc. of 24 hour glucose broth culture No. 365 a injected into wing vein. 10.40 a.m. Red cells 4,700,000; no free nuclei present. 11.50 a.m. Pigeon drooping. Red cells 3,880,000, free nuclei appearing. 2 p.m. Red cells 2,432,000; many free nuclei. 3.45 p.m. Dying. Red cells 1,520,000. Immediate autopsy showed some but not numerous bacilli in blood; cytoplasm of the red cells stains weakly; blood serum reddish brown in color.

(*b*) Similar to (*a*). In 8 hours, when death occurred, the red cells had fallen from about 5,000,000 per c.mm. to 1,000,000. The autopsy findings were typical.

In other words, the injection of 0.5 cc. of a broth culture of active *Bacillus welchii* intravenously into pigeons causes extensive blood destruction and death in periods of from 6 to 8 hours. These results are now to be contrasted with pigeons in which the culture is injected into the pectoral muscles.

Experiment 10.—(*a*) Pigeon. Red cells, 4,500,000 per c.mm. 9.30 a.m. 0.5 cc. of glucose broth culture No. 365 a injected into breast muscle. 12 m. Drooping. 3.30 p.m. Red cells 4,600,000; no free nuclei. 4.45 p.m. Dying. Red cells 4,450,000. Autopsy showed local infection; no bacilli in blood.

(*b*) Similar to (*a*) except that Culture 617 d was employed. At the outset the red cell count was 4,450,000 per c.mm. 6 hours later, when the animal was dying, it was 4,435,000. The autopsy findings were characteristic.

These experiments several times repeated were always consistent. Intravenous injections of broth cultures are attended by extensive blood destruction and death; intramuscular injections of like doses cause death with equal certainty and rapidity but no blood destruction. Hence the blood destruction cannot be the determining factor of the lethal action. The essential toxic agent appears now not to be an acid and not an hemolysin. The next experiments relate to its filterability.

Up to the present the fluid cultures described were not wholly free from the bacilli. To remove the bacilli entirely, in order to test the toxicity of the sterile fluid, filtration through a Berkefeld N candle was resorted to. The first tests were made with filtrates obtained from ordinary glucose broth cultures. They indicated merely a low degree of toxicity for rabbits and pigeons.

Experiment 11.—A 24 hour glucose broth growth of Culture 617 d was employed as follows:

(*a*) Rabbit; weight 1,475 gm. 10 cc. of the supernatant fluid obtained by centrifugation for 20 minutes, not quite clear, and having an acidity of 4.2 per cent were injected intravenously. The animal had a severe convulsion and died almost immediately. The blood was extensively destroyed.

(*b*) Rabbit; weight 1,425 gm. 11 cc. of a Berkefeld filtrate, having an acidity of 3.9 per cent, were injected intravenously. No symptoms.

(*c*) Rabbit; weight 1,575 gm. 3 p.m. 10 cc. of the clear fluid obtained by centrifugation for 40 minutes and having an acidity of 4.5 per cent were injected intravenously. For an hour there was respiratory distress, which passed off. 7 p.m. Died. Kidneys chocolate colored Another part of this fluid first filtered, then injected into a normal rabbit, produced no effect.

This experiment shows that not only does filtration reduce the toxicity, but long centrifugation does also. The difference is not caused by the reduction in acidity observed in the filtered fluid, since neutralization of the centrifugate with sodium hydroxide did not affect its activity. The injection of the filtrate in amounts of 8 cc. into the breast muscles of pigeons caused temporary drooping but no local lesion or other severe effect. No distinction in action was

noted in the different cultures. The conclusion to be drawn from these experiments is that a certain kind of toxic product is developed in glucose broth cultures, but that prolonged centrifugation and filtration tend to remove it from the fluid. Since the action is so rapid, it does not seem probable that there is any actual relationship between the bacilli as such still remaining in the centrifuged fluid and the poisonous agent. The latter is not an ordinary acid and appears to be an hemolysin.

The next experiments throw an entirely different light on the toxin-producing property of *Bacillus welchii*. It is obvious that cultures in glucose broth in no way represent the conditions occurring during local infections in man and animals. Hence these were simulated in the following manner.

To plain beef infusion broth in 10 cc. quantities in test-tubes were added several fragments of sterile skeletal muscle of the pigeon or rabbit. The tubes, having been proved sterile, were inoculated with *Bacillus welchii* and overlaid with paraffin oil and enclosed in a vacuum jar from which the oxygen was exhausted. After an incubation of from 18 to 24 hours, the fluid was centrifuged and filtered through a Berkefeld N candle. The filtrate was always free of the bacilli. This product proved highly toxic for pigeons, guinea pigs, and rabbits, and, what should be emphasized, gave rise to inflammatory and other local lesions resembling closely those caused by the bacilli themselves. While Culture 617 d, the most virulent of all, yielded the most active filtrate, yet all five cultures gave toxic products. The degree and manner of the action of the toxic filtrates are indicated by the following illustrative protocols.

Experiment 12.—(*a*) Guinea pig. 3 p.m. 2 cc. of the Berkefeld filtrate of an 18 hour pigeon muscle broth culture of Culture 617 d were injected beneath the skin of the right thigh. The next morning at 8 a.m., the entire leg was swollen and the joints held stiffly; the scrotum was also edematous. The animal crouched in the corner of the cage. 3 days later the hair was loose, and the tissues were sloughing. Death occurred during the night. The autopsy showed disorganization of the muscles of the right leg and adjacent abdominal wall. Cocci, but no gas bacilli were present.

Doubtless the filtrate acted upon the skin and underlying muscles, inducing inflammation and necrosis, after which pyogenic cocci and

other bacteria entered the injured tissues and produced the sloughing. A dose of 1 cc. of the filtrate caused a similar but less severe lesion, from which the guinea pig slowly recovered after healing of the defect.

(*b*) Rabbit. 2 cc. of the same lot of toxin used in (*a*) were injected under the skin of the right thigh at 4.30 p.m. The next day the skin over the leg and the right scrotal sac was highly edematous. 5 days later, the edema subsided, leaving a dry necrotic area behind, which finally was thrown off and became healed.

The local effect, therefore, in the rabbit is similar to although less severe than that in the guinea pig. The effect in the rabbit of an intravenous injection of the same lot of toxin is to produce acute blood destruction and death. Thus a rabbit having a red cell count of 5,400,000 was given 1 cc. of the toxin at 10 a.m. At 11 a.m. the cells numbered 4,250,000; at 12.30 p.m., 2,550,000; at 4 p.m., 1,500,000; at 5 p.m., 1,000,000. Death took place during the night. The kidneys were chocolate colored and the urine dark.

(*c*) Pigeon. 12 m. 0.2 cc. of a similar toxin was injected into the right breast muscles. The next morning at 8 o'clock there was widespread edema of the injected site. 24 hours later the edema was subsiding. 3 days later the swelling had disappeared. On etherization and autopsy, an extensive necrotic focus was found in the injected pectoral muscles; the muscles of the opposite side were normal.

The local reaction of the pigeon to small doses of the toxic filtrate resembles that of the guinea pig to far larger doses. When 0.3 cc. of the filtrate was injected, the edema developed very quickly and death occurred in about 4 hours—even more quickly, therefore, than from massive bacillary infection. The injected muscle was already friable. No effect is produced in the red blood corpuscles. When, however, the filtrate is injected into the wing vein, hemolysis results. Thus a pigeon having a blood count of 4,280,000 was given 0.25 cc. of neutral filtrate at 9.30 a.m. At 10.30 a.m., the cells were unchanged in number and no free nuclei occurred. At 4.30 p.m. the cells numbered 3,725,000, and free nuclei were found. The next day at 1 p.m. the cells numbered 800,000, and there was marked air hunger. Death took place at 5 p.m.

This experiment, which was repeated several times, shows that *Bacillus welchii*, when the conditions of growth are suitable, yields toxic products of high potency. These products produce two sets of effects according to the manner of their injection into animals: (*a*) hemolysis, in which they resemble the effects arising from ordinary glucose broth cultures; (*b*) inflammation and necrosis of subcutaneous tissue and muscles, in which they resemble the effects produced by the bacilli themselves. Even moderate quantities of the toxic filtrate locally injected may also bring about rapid death of pigeons.

It is now possible to answer the question placed at the head of this section of our paper. The cause of death in *Bacillus welchii* infection is not a blood invasion of the microorganisms and not acid intoxication, but an intoxication with definite and very potent poisons produced in the growth of the bacilli in the tissues of the body. This poison is readily produced in broth in the test-tube in the presence of sterile non-denatured muscle. To obtain it in quantity only minimal quantities of glucose (0.1 per cent) should be added to the broth, and the incubation of the anaerobic cultures should not exceed 24 hours. The poison or toxin is a complex of an hemolysin and another poisonous body. The latter is the more toxic, since it may bring about death under conditions in which no blood destruction takes place.

The Toxic Product.

Thermolability.—The toxicity of the filtered fluid is destroyed by heating 30 minutes at 70°C. in sealed tubes and is greatly diminished by similar heating at 62°C. The fluid subjected to the latter treatment no longer causes death in pigeons, even when large doses are injected, although a degree of necrosis of the muscles still results. A test made to determine the point indicates also that the substances exerting the toxic effects do not dialyze through collodion membranes.

Antigenic Properties.—The next step taken was that of determining whether the toxic product would act as an antigen. Two sets of tests were made: (*a*) the setting up of active immunity of the pigeon; (*b*) the production of an antiserum in the rabbit.

The former is difficult to accomplish because of the necrosis caused even by sublethal injections into the pectoral muscles of pigeons. However, by giving three carefully graded injections at weekly intervals, the animals may be kept in fair condition. 1 week after the last injection, the pigeons bore two lethal doses of the toxic filtrate without reaction.

The latter is accomplished with less difficulty. Large male rabbits were employed. 2 cc. of a neutralized filtrate of Culture 617 d were injected beneath the skin of the inner aspect of the thigh. This was followed by edema involving the scrotum. The edema subsided in a few days, leaving the scrotal skin necrotic and dry. 10 days after the first injection a second one was given on the opposite side. The effects were the same as the first. A third injection of 3.5 cc. was given on the right side after a similar interval. No reaction followed. The rabbit was now bled and a series of neutralizations performed, as shown in Table I. The toxic filtrate was mixed with the serum from the immunized rabbit or normal rabbit and injected immediately into the pectoral muscles of the pigeon or subcutaneously into the rabbit and guinea pig.

The table shows that the blood of a rabbit which has received three injections of a toxic filtrate from a given culture is capable of neutralizing not only that particular filtrate, but the filtrate from four other cultures as well. The neutralization is effective against the filtrate obtained from several distinct cultures and for the three species of animals—pigeon, rabbit, and guinea pig—employed.

Moreover, the neutralization is not only for the toxic substance causing inflammation and necrosis of the local tissues, but also for the specific hemolysin contained in the filtrates. This is an important point, since it controverts the notion that the blood destruction results from acids produced in course of growth of the bacilli.

Experiment 13.—(*a*) Pigeon. Red cells 4,250,000; no free nuclei. 2.10 p.m. Injected into wing vein mixture of 1 cc. of toxic filtrate of No. 617 d and 1 cc. of normal rabbit serum. 3.40 p.m. Red cells 1,312,000; many free nuclei. 3.40 p.m. Death.

(*b*) Pigeon. Red cells 4,500,000. 11.45 a.m. Mixture of 1 cc. of toxic filtrate of No. 617 d and 1 cc. of immune rabbit serum injected into wing vein. 1.45 p.m. Red cells 4,264,000. 10 a.m. next day. Red cells 4,300,000. No symptoms appeared.

TABLE I.

Hr. of injection.	Animal injected.	Toxic product.		Mixed with immune or normal rabbit serum.		Local reaction.	Final result.
		Quantity.	Source.				
a.m.		*cc.*		*cc.*			
9	Pigeon.	1	617 d	0.5	Immune.	None.	Survived.
9	"	1	617 d	0.5	Normal.	In 2 hrs. extensive edema.	Died, 2.10 p.m.
9	"	3	365 a	1.0	Immune.	None.	Survived.
9	"	3	365 a	1.0	Normal.	In 3 hrs. extensive edema.	Died, 4.10 p.m.
9	"	3	669 b	1.0	Immune.	None.	Survived.
9	"	3	669 b	1.0	Normal.	In 4½ hrs. muscle greatly swollen.	Died, 1.45 p.m.
9	"	3	P-50	1.0	Immune.	None.	Survived.
9	"	3	P-50	1.0	Normal.	In 5 hrs. muscle greatly swollen.	Died, 2.30 p.m.
9	"	4	386 cd	1.0	Immune.	None.	Survived.
9	"	4	386 cd	1.0	Normal.	In 5 hrs. muscle swollen.	Died, 2.45 p.m.
10	Rabbit.	2	617 d	1.0	Immune.	None.	Survived.
10	"	2	617 d	1.0	Normal.	Extensive scrotal edema.	Recovered.
10	Guinea Pig A.	2	617 d	1.0	Immune.	None.	Survived.
10	" " B.	2	617 d	1.0	Normal.	Extensive swelling of leg and scrotum.	Necrosis. Died on 7th day.

Neutralizing Proportions.—The next experiment was designed to determine the minimal lethal dose of the toxic filtrate and the necessary neutralizing quantity of immune serum for that dose. This having been ascertained, an experiment was conducted to decide whether the neutralization took place equally in multiple proportions.

Pigeons were employed for the tests. The minimal lethal dose of the toxic filtrate employed proved to be 0.3 cc., from which death resulted in about 8 hours. The perfectly neutralizing quantity

of the immune serum for this dose was 0.2 cc. When mixed together and injected into the pectoral muscles no reaction followed. The minimal protective dose of the immune serum was much smaller; namely 0.05 cc. But with this dose considerable local reaction manifested itself.

The experiment with multiple proportions of toxic filtrate and immune serum was made with twenty-five doses of each. Hence 7.5 cc. of the toxic filtrate and 5.0 cc. of the immune serum were mixed and the entire volume was then injected into the breast muscles of each of two pigeons. No signs of intoxication developed, and aside from slight local edema in one of the pigeons, no symptoms whatever appeared. From this it was concluded that the toxic filtrate and antitoxic rabbit blood neutralized each other perfectly in multiples of the single doses. In this respect the two resemble the corresponding toxins and antitoxins of *Bacillus diphtheriæ* and *Bacillus tetani*.

Protective and Curative Properties.

The experiments described having clearly shown that the toxic products of the growth of *Bacillus welchii* exhibit antigenic activities and readily give rise to the formation of active antitoxic substances, the obvious next step was to determine whether the immune serum developed possessed protective and curative properties. Two sets of tests bearing on these questions have been made.

In one, vegetative bacilli have been injected into the breast muscles of pigeons mixed with or followed by the immune serum. The result is to prevent or reduce the pathogenic effects otherwise produced. Normal rabbit serum has no such power of control. These experiments will be published in detail later.

In the other, the object was to imitate conditions of natural infection in man with a view to preventing infection from arising. For this purpose, the bacilli were cultivated by Dunham's method so as to obtain spores. Bits of gauze were impregnated with the sporulating cultures and thrust into the breast muscle of the anesthetized pigeons with a small hemostat. The wound at once filled with blood and became sealed. Hence the conditions of a foreign body carrying active spores of the gas bacilli imbedded in muscle tissue

and protected from access of air as occurring in man were reproduced on a small scale. The test proved a severe one. Five pigeons were employed in a series. One only was treated with the immune serum, the other four serving as controls. This plan was adopted to remove the fallacy of an accidental survival of the treated animal. 2.0 cc. of the immune serum were injected, partly about the wound, partly in the opposite breast. The four inoculated but untreated pigeons developed typical local lesions and succumbed in 20 to 40 hours after inoculation. The treated animal never showed any local or general symptoms, survived, and the wound healed about the foreign body.

The experiments briefly reported in this section of the paper seem to possess considerable importance. They indicate, indeed, that in *Bacillus welchii* infection in nature the development of the spores into vegetative bacilli may be prevented by a protective inoculation of an antitoxic serum, and also that the vegetative bacilli may be deprived by such a serum of their toxic products, which now appear to be their real offensive instrument. We are confronted, therefore, not only with a new point of view regarding the manner of the pathogenic action of the Welch group of bacilli but also with a new means of combating their pathogenic effects.

DISCUSSION.

The experiments presented appear to admit of one interpretation only; namely, that the Welch bacilli, under suitable conditions of growth, produce an active exotoxin, to which their pathogenic effects are ascribable. The toxic product, moreover, acts upon the local tissues and the blood in a manner identical with the action of the cultures. With the toxic product animals may be immunized actively and yield an immune serum which neutralizes the toxin perfectly and in multiple proportion. The toxic bodies would seem to be at least two in number: one causing blood destruction, hence an hemolysin, and the other acting locally on the tissues and blood vessels, causing edema and necrosis and probably exerting general toxic action in addition. The part each plays in bringing about the lethal effect seems to be determined by the manner of inoculation: to bring out the

hemolytic action intravenous injection is indicated; to bring out the locally destructive action, subcutaneous or intramuscular injection is required.

This conception of the manner of pathogenic action of the Welch bacilli is totally different from any view previously held. It is true that others have attributed the general symptoms in *Bacillus welchii* infection to an intoxication; but the poisoning meant was one ascribed on the one hand to decomposition products of the infected tissues (E. Fraenkel) and on the other to ordinary endotoxin absorption (Metchnikoff, Korentchewsky, Kamen, Herter, Passini). Other views have also been expressed and insisted upon, and they would ascribe the locally destructive effects of the bacilli to mechanical action (Taylor) or to the production of fatty acids which also through the setting up of an acidosis bring about a lethal termination (McCampbell, Stewart and West, Wright).

Reference will be made only to the views expressed by recent writers who have encountered gaseous gangrene in connection with gun-shot wounds of the great war. Thus Weinberg,[6] who believes that the gas-producing bacilli do not cause the gangrene, but that the condition precedes the infection, has obtained toxic and antitoxic products from various anaerobic bacteria isolated from gangrenous wounds. With *Bacillus perfringens* (of the *Bacillus welchii* group) he has prepared an antibacterial serum, but he failed to detect either the exotoxin or its corresponding antiserum.

Kenneth Taylor[7] considers that gaseous gangrene is the result of the mechanical action of the gas produced in a local focus of developing saprophytic bacteria. He specifically draws the distinction between *Bacillus tetani* and *Bacillus welchii* infections, since with the former the toxin is the active factor, while with the latter the mechanical effect of the gas is paramount. The mechanical process he conceives to be as follows: *Bacillus welchii* attacks the carbohydrates of muscular tissue and produces a large volume of gas, which, being unable to escape from the tissues, exerts pressure upon the blood vessels, impeding the circulation so that necrosis results. The necrotic tissue is invaded by putrefactive bacilli which disorganize it.

Sir Almroth Wright[8] holds that *Bacillus welchii* operates through the production of an acid condition of the blood and tissues, through which the antitrypsin is diminished. Because of this diminution, tryptic digestion of the proteins is permitted and the bacilli are thus provided with a highly favorable medium of growth, so that multiplication becomes explosive in nature. The intoxication following is in fact an acidemia.

---

[6] Weinberg, M., *Proc. Roy. Soc. Med.*, 1916, ix, Occas. Lect., 119.

[7] Taylor, K., *Bull. Johns Hopkins Hosp.*, 1916, xxvi, 297; *J. Path. and Bacteriol.*, 1916, xx, 384.

[8] Wright, A. E., *Proc. Roy. Soc. Med.*, 1916–17, x, Occas. Lect., 1.

Conradi and Bieling[9] distinguish two phases of action of the bacilli. In the first or fermentation phase, the carbohydrates are attacked, and lactic, butyric, propionic, and succinic acids are formed, which are the immediate causes of the edema and necrosis of the tissues. In the second or saprophytic stage, the spore-bearing organisms appear and appropriate the dead tissue, giving rise to putrefaction and consequent intoxication.

These brief extracts readily indicate not only the wide diversity of opinion held by recent students of the pathogenesis of gas bacillus infection in man, but show also how remote the conceptions are from that of a specific pathogenetic process, due to the action of particular toxic substances, which is the basis of the conviction derived from the experiments described by us. According to our view, infection by *Bacillus welchii*, like infection by *Bacillus tetani*, essentially resolves itself into an intoxication, in which an exotoxin yielded by the multiplying organisms constitutes the chief danger. The two conditions differ, however, with respect to the local effects produced on the tissues, since the tetanus toxin does not possess inflammatory and necrotizing properties. The Welch bacilli, therefore, grow more abundantly and produce wide destruction of tissue, in which process they are soon assisted by the usual pyogenic microorganisms, which quickly obtain a foothold in the disorganized structures.

SUMMARY.

Five cultures of *Bacillus welchii* have been studied and compared. Four came from infected wounds in the western theatre of war, and one was obtained from a personal article of clothing. Each culture possesses the essential characteristics ascribed to that group of bacteria.

The infectious processes caused by the five cultures in rabbits, guinea pigs, and pigeons, are local in character; and very few or no bacilli enter or are found in the general blood stream during life or immediately after death.

Glucose broth cultures, injected intravenously, are fatal to rabbits. Death occurs almost immediately or after a few hours. Agglutinative bacterial emboli have been ruled out as the cause of death,

[9] Conradi, H., and Bieling, R., *Münch. med. Woch.*, 1916, lxiii, 1608.

as has been an acid intoxication. The fluid part of the culture acts in the same manner as the full culture and irrespective of neutralization with sodium hydroxide.

The full cultures and supernatant fluid are hemolytic when injected directly into the circulation of rabbits and pigeons, and the acute death produced may be ascribed to a massive destruction of red corpuscles. The passage of the fluid portion of glucose broth cultures through Berkefeld filters reduces materially the hemolytic and poisonous effects.

Cultures of the Welch bacilli in plain broth to which sterile pigeon or rabbit muscle is added are highly toxic, and the toxicity is not noticeably diminished by Berkefeld filtration. The filtrates are hemolytic when injected intravenously and inflaming and necrotizing when injected subcutaneously and intramuscularly. The local lesions produced in the breast muscles of the pigeon closely resemble those caused by infection with the bacilli.

The toxicity of these filtrates is not affected by neutralization with sodium hydroxide, but is materially reduced by heating to 62°C. and entirely removed by heating to 70°C. for 30 minutes.

Successive injections of carefully graded doses of this toxic filtrate in pigeons and rabbits give rise to active immunity. The blood taken from the immunized rabbits is capable of neutralizing the toxic filtrate *in vivo* and *in vitro*. The filtrate has therefore been designated as toxin and the immune serum as antitoxin.

The antitoxin neutralizes the toxin in multiple proportions. Hence the latter would seem to possess the properties of an exotoxin. Moreover, it neutralizes the hemolytic as well as the locally injurious toxic constituent.

Antitoxic serum prepared from a given culture of *Bacillus welchii* is neutralizing for the toxins yielded by the other four cultures of that microorganism.

The antitoxin is protective and curative against infection with the spore and the vegetative stages of *Bacillus welchii* in pigeons. The limits of the protective and curative action are now under investigation.